

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

JC07 Rec'd PCT/PTO T 6 APR 2001

0652.2200000/EKS/SEZ

U.S. APPLICATION NO. (IF KNOWN, SEE 37 C.F.R. § 1.5)

To Be Assigned

097807512

INTERNATIONAL APPLICATION NO.

PCT/EP99/07832

INTERNATIONAL FILING DATE

October 15, 1999

PRIORITY DATE CLAIMED

October 16, 1998

TITLE OF INVENTION

Camel, An Alternative Translation Product of the Tumor Antigen L

APPLICANT(S) FOR DO/EQ/US

Schrier et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made, however, the time limit for making such amendments has NOT expired
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included
13. ☒ A FIRST preliminary amendment
  - ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Authorization to Treat a Reply as Incorporating an Extension of Time Under 37 C.F.R. § 1.136(a)(3).

U.S. APPLICATION NO. (if known) 37 CFR 1.50 To Be Assigned <b>09/807512</b>		INTERNATIONAL APPLICATION NO. PCT/EP99/07832		ALTERNATIVE DISCLOSURE NUMBER 0652.2200000	
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17. <input checked="" type="checkbox"/> The following fees are submitted.			CALCULATIONS PTO USE ONLY		
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO <span style="float: right;"><b>\$1000.00</b></span>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO <span style="float: right;"><b>\$860.00</b></span>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO <span style="float: right;"><b>\$710.00</b></span>  International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) <span style="float: right;"><b>\$690.00</b></span>  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) <span style="float: right;"><b>\$ 100.00</b></span>					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT</b>			=	\$ 860.00	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).			\$ 130.00		

Claims	Number Filed	Number Extra	Rate		
Total Claims	25 - 20 =	5	X <b>\$18.00</b>	\$90.00	
Independent Claims	2 - 3 =	0	X <b>\$80.00</b>	\$ -0-	
Multiple dependent claim(s) (if applicable)			+ <b>\$270.00</b>	\$ -0-	
<b>TOTAL OF ABOVE CALCULATIONS</b>				=	\$ 1080.00
<input type="checkbox"/> Applicant claims small entity status Sec 37 CFR 1.27 The fees indicated above are reduced by 1/2				\$-0-	
<b>SUBTOTAL</b>				=	\$1080.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ -0-	
<b>TOTAL NATIONAL FEE</b>				=	\$ 1080.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) <b>\$40.00</b> per property				\$ -0-	
<b>TOTAL FEES ENCLOSED</b>				=	\$ 1080.00
				Amount to be refunded:	\$
				charged:	\$


a. ☒ A check in the amount of **\$1080.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **19-0036**. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO <b>STERNE, KESSLER, GOLDSTEIN &amp; FOX P.L.L.C.</b> 1100 New York Avenue, N.W., Suite 600 Washington, D.C. 20005-3934	 Eric K. Steffe NAME <b>36,688</b> REGISTRATION NUMBER
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097807512

JC02 Rec'd PCT/PTO 16 APR 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

*In re* application of:

Schrier *et al.*

Appl. No. *To Be Assigned*  
(National Phase of International Appl. No.  
PCT/EP99/07832, filed October 15, 1999)

Filed: April 16, 2001

For: **Camel, An Alternative  
Translation Product of the Tumor  
Antigen Lage-1**

Confirmation No.:

Art Unit: *To Be Assigned*

Examiner: *To Be Assigned*

Atty. Docket: 0652.2200000/EKS/SEZ

**Preliminary Amendment**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

In advance of substantive examination in the above identified matter, please  
amend the specification as follows:

- (A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;
- (B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.115 and MPEP 714; and
- (C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net

addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

***Amendments***

***In the Specification:***

Please insert the following sections into the specification:

Please insert the following statement at page 1 after the title:

--CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/EP99/07832, filed October 15, 1999.--;

At page 1, after the Cross Reference and before line 1 of the text, please insert:

--BACKGROUND OF THE INVENTION

Field of the Invention--;

At page 1, before line 4, please insert --Related Art--.

At page 2, before line 3, please insert --BRIEF SUMMARY OF THE INVENTION--;

At page 2, before line 5, please insert --DETAILED DESCRIPTION OF THE INVENTION--.

At page 11, line 7, please delete the phrase --Brief description of the Figures-- and insert therefor the phrase --BRIEF DESCRIPTION OF THE DRAWINGS--.

At page 14, after line 5, please insert --EXAMPLES--.

At page 34, line 1, please delete --Claims-- and insert therefor --WHAT IS CLAIMED IS:--.

At page 36, line 1, please insert --ABSTRACT--;

At page 36, line 3, please insert the following paragraph: --The tumor-associated antigen CAMEL and DNA encoding the antigen are provided. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.--.

***In the Claims:***

Please cancel claims 1-14 without prejudice or disclaimer.

Please add the following claims:

15. (New) An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.
16. (New) An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.
17. (New) The isolated polypeptide of claim 16 comprising SEQ ID NO: 11.
18. (New) The isolated polypeptide of claim 16 comprising SEQ ID NO: 12.
19. (New) The isolated polypeptide of claim 16 comprising SEQ ID NO: 24.
20. (New) The isolated polypeptide of claim 16 comprising SEQ ID NO: 25.
21. (New) The isolated polypeptide of claim 16 comprising SEQ ID NO: 26.

22. (New) A composition comprising the polypeptide of claim 15 and a pharmaceutically acceptable carrier.
23. (New) A composition comprising the polypeptide of claim 16 and a pharmaceutically acceptable carrier.
24. (New) An isolated nucleic acid molecule encoding the polypeptide of claim 15.
25. (New) The nucleic acid molecule of claim 24 comprising the coding region of SEQ ID NO:1.
26. (New) The isolated nucleic acid molecule of claim 25 comprising SEQ ID NO. 1.
27. (New) An isolated nucleic acid molecule encoding the polypeptide of claim 16.
28. (New) A composition comprising the nucleic acid molecule of claim 24 and a pharmaceutically acceptable carrier.
29. (New) A composition comprising the nucleic acid molecule of claim 27 and a pharmaceutically acceptable carrier.
30. (New) A vector comprising the nucleic acid molecule of claim 24.
31. (New) A vector comprising the nucleic acid molecule of claim 27.
32. (New) A host cell comprising the vector of claim 30.

33. (New) A host cell comprising the vector of claim 31.
34. (New) A method for inducing a cytotoxic T lymphocyte response *in vivo* comprising administering to an individual in need thereof an effective amount of the polypeptide of claim 15 or an effective amount of a polynucleotide encoding said polypeptide.
35. (New) A method for inducing a cytotoxic T lymphocyte response *in vivo* comprising administering to an individual in need thereof an effective amount of the polypeptide of claim 16 or an effective amount of a polynucleotide encoding said polypeptide.
36. (New) An *ex vivo* method for treating an individual comprising
- (a) incubating cytotoxic T lymphocyte (CTL) precursor cells obtained from the individual with antigen presenting cells and the polypeptide of claim 15;
  - (b) allowing said precursor cells to mature and expand to effector CTLs; and
  - (c) readministering said effector CTLs to the individual.
37. (New) An *ex vivo* method for treating an individual comprising
- (a) incubating cytotoxic T lymphocyte (CTL) precursor cells obtained from the individual with antigen presenting cells and the polypeptide of claim 16;
  - (b) allowing said precursor cells to mature and expand to effector CTLs; and
  - (c) readministering said effector CTLs to the individual.

38. (New) An *ex vivo* method for treating an individual comprising administering to the individual cells transfected with the nucleic acid molecule of claim 24.
39. (New) An *ex vivo* method for treating an individual comprising administering to the individual cells transfected with the nucleic acid molecule of claim 27.



**Remarks**

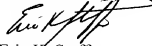
Upon entry of the foregoing amendment, claims 15- 39 are pending in the application, with claims 15 and 16 being the independent claims. Claims 1-14 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 15-39 are sought to be added. By entry of the foregoing amendment, Applicants have amended the international application to place the specification and claims into proper format for U.S. practice. Support for new claims 15-39 is found, *inter alia*, in original claims 1-14, specification page 7, lines 12-30; page 8, lines 1-30; page 9, lines 1-30; page 10, lines 1-7 and elsewhere throughout the specification. Hence, no new matter has been added by the amendment and entry and consideration of the same is respectfully requested.

**Conclusion**

It is respectfully believed that the present application is in condition for examination. Early notice to this effect is earnestly solicited. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Eric K. Steffe  
Attorney for Applicants  
Registration No. 36,688

Date: April 16, 2001  
1100 New York Avenue, N.W.  
Suite 600  
Washington, D.C. 20005-3934  
(202) 371-2600

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**Version with markings to show changes made**

Claims 1-14 have been canceled.

Claims 15-39 are newly added.

A statement at page 1, after the title, has been added.

Two statements at page 1, before line 1 of the text, have been added.

A statement at page 1, before line 4, has been added.

A statement at page 2, before line 3, has been added.

A statement at page 2, before line 5, has been added.

A statement at page 11, line 7, has been deleted and a statement inserted therefor as follows:

[Brief description of the Figures] **BRIEF DESCRIPTION OF THE DRAWINGS**

A statement at page 14, after line 5, has been added.

A statement at page 34, line 1, has been deleted and a statement inserted therefor as follows: [Claims] **WHAT IS CLAIMED IS:**

A statement at page 36, line 1, has been added.

A paragraph at page 36, line 3, has been added.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Schrier *et al.*

Appl. No. 09/807,512

Filed: April 16, 2001

**For: Camel, An Alternative Translation  
 Product of the Tumor  
 Antigen-Lage I**

Confirmation No. 9121

Art Unit: *To be assigned*

Examiner: *To be assigned*

Atty. Docket: 0652.2200000/EKS/Y-W

**Supplemental Preliminary Amendment and  
 Submission of Substitute Sequence Listing  
 Under 37 C.F.R. § 1.825(a)**

Commissioner for Patents  
 Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements, dated November 8, 2001, in the above-identified matter, and in compliance with 37 C.F.R. § 1.825(a), Applicants submit the following Amendments and Remarks. This Amendment is provided in the following format:

- (A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;
- (B) Starting on a separate page, appropriate remarks and arguments; and
- (C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

As a reply to this Notification to File Missing Requirements was due on January 8, 2002, a Petition for Extension of Time is enclosed herewith.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

### *Amendments*

#### *In the Specification:*

Please amend the specification as follows:

At page 1, after the title, please delete the first paragraph of cross reference statement, which was inserted previously in the Preliminary Amendment filed on April 16, 2001 and replace it with the following paragraph:

#### CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application PCT/EP99/07832, filed October 15, 1999, and published under PCT Article 21(2) in English as WO 00/23584 on April 27, 2000.

At the end of the application, please cancel the existing Sequence Listing and replace it with the substitute Sequence Listing appended hereto, and insert the same.

**Remarks**

Upon entry of the foregoing amendment, Applicants have amended the specification only to comply with the requirements of C.F.R. 37 §1.78(a), and to cancel the existing sequence listing and direct the entry of the substitute sequence listing at the end of the application. The substitute sequence listing is identical to the existing sequence listing. Hence, no new matter has been added by the amendment and entry and consideration of the same is respectfully requested.

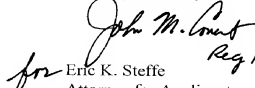
In accordance with 37 C.F.R. §1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. §1.821(f), the paper copy of the sequence listing and the computer readable copy of the sequence listing submitted herewith in the above application are the same.

It is respectfully believed that the present application is in condition for substantive examination. Early notice to this effect is earnestly solicited. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

  
for Eric K. Steffe  
Attorney for Applicants  
Registration No. 36,688  
Reg No 38,759

Date: April 8, 2002

1100 New York Avenue, N.W.  
Washington, D.C. 20005-3934  
(202) 371-2600

**Version with markings to show changes made*****In the Specification:***

The specification was amended as follows:

At page 1, after the title, the first paragraph of the cross reference statement, which was inserted previously in the Preliminary Amendment filed April 16, 2001, was replaced with the following paragraph:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application PCT/EP99/07832, filed October 15, 1999, and published under PCT Article 21(2) in English as WO 00/23584 on April 27, 2000.

The existing sequence listing has been canceled and replaced with the substitute sequence listing attached herewith, which was inserted at the end of the application.

## CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1

The present invention relates to the field of cancer therapy, more specifically to tumor-associated antigens.

Cytotoxic T lymphocytes (CTLs) play an important role in the defense against melanoma. Melanoma-specific CTL clones have been obtained from either tumor infiltrating lymphocytes (TIL) *in vitro* stimulated with cytokines, or from peripheral blood mononuclear cells (PBMC) cultured with (autologous) tumor cells. T cell responses against tumor cells are enhanced by cytokine transfection of the tumor cells, both in animal models and in *in vitro* human culture systems. (van Elsas et al., 1997; Gansbacher et al., 1990; Tepper et al., 1989; Fearon et al., 1990; Dranoff et al., 1993)

The antigens recognized by the tumor-specific T cells become better defined by the development of molecular cloning techniques. These T cell targets can be divided in three groups: 1) tumor-specific antigens, not expressed in healthy tissues, except testis and placenta (e.g., MAGE, BAGE, GAGE, NY-ESO-1, LAGE-1); 2) antigens that are lineage-specific and expressed in both melanoma and melanocytes (e.g., MART-1/ Melan-A, gp100, tyrosinase) and 3) unique, mutated antigens (e.g.,  $\beta$ -catenin, CDK4, MUM-1) (reviewed by Van den Eynde and Brichard, 1995).

By means of Representational Difference Analysis (RDA), a PCR-based method that has been used to identify genes with tissue-specific or tumor-specific expression, the LAGE-1 and NY-ESO1 genes were identified as being tumor specific by screening cDNA libraries from melanoma cell lines with a primer from a cDNA clone enriched in melanoma-specific sequences (Lethe et al., 1998).

NY-ESO-1 is a gene originally identified by SEREX technology (Chen et al., 1997). It was shown to have two different reading frames (DNA sequences and derived protein sequences given in SEQ ID NO: 7 - 10), translation

products of which were shown to contain epitopes of tumor specific T-cells (Jäger et al., 1998; Wang et al., 1998).

It was an object of the present invention to provide a novel tumor-associated antigen.

- 5 To solve the problem underlying the invention, melanoma cell line 518A2 and its IL-2- or GM-CSF-transfectants were compared for their CTL stimulating capacity *in vitro*. Stimulation of autologous PBMC with the IL-2 producing melanoma cells resulted in a melanoma-specific CTL response (van Elsas et al., 1997). CTL clones derived from this culture recognized,  
10 besides autologous melanoma cell lines, also a panel of HLA-A\*0201 positive melanoma cell lines, but were not reactive with normal melanocytes. Although 518A2 was shown to express a number of antigens previously identified to be recognized by anti-melanoma CTL (van Elsas et al., 1996), the CTL clones available recognize a new melanoma-specific  
15 antigen that is immunodominant in 518A2.

- In the experiments of the present invention, the target structure that was recognized by one of these CTL clones was fully characterized and named CAMEL (CTL-recognized Antigen on Melanoma). These sequences are described in the attached sequence listing as SEQ ID NO: 1 and SEQ ID  
20 NO: 2.

It was surprisingly found that CAMEL is encoded by a reading frame of the LAGE-1<sup>s</sup>-cDNA (SEQ ID NO:3) that is distinct from that encoding the putative LAGE-1 protein (SEQ ID NO: 4). (This reading frame is designated ORF-1.)

- 25 In the present invention, a cDNA clone was identified that lacks the first 84 bp of the LAGE-1<sup>s</sup> sequence (SEQ ID NO: 3) which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in this clone (4H8) is the ATG at position 94 of LAGE-1<sup>s</sup> (SEQ ID NO: 3), which is however, not in frame with



the first ATG at position 54. Therefore, the CAMEL protein (SEQ ID NO: 2) translated from the 4H8 cDNA clone is different from the putative LAGE-1<sup>S</sup> protein (SEQ ID NO: 4).

5 In a first aspect, the present invention is directed to the tumor-associated antigen CAMEL (SEQ ID NO: 2) which is encoded by an isolated DNA molecule with the sequence as defined in SEQ ID NO: 1.

The coding sequence of CAMEL corresponds to the ORF-1 of LAGE-1 cDNA (Lethe et al., 1997; WO 98/32855).

10 In the present invention „ORF-1“ is defined as the open reading frame starting with ATG at position 94 of SEQ ID NO:3 (LAGE-1<sup>S</sup>), which corresponds to position 10 in SEQ ID NO: 1 (CAMEL), to position 96 in SEQ ID NO: 5 (LAGE-1<sup>L</sup>)

15 In a further aspect, the present invention relates to immunogenic (poly)peptides derived from CAMEL. A first group of peptides is selected from peptides inducing a humoral immune response (induction of antibodies). Such peptides are selected by randomly choosing continuous stretches of amino acids (at least 12-15), applying them to an individual and confirming the generation of antibodies by standard immunological assays, e.g. ELISA. This group of immunogenic (poly)peptides also encompasses  
20 the entire CAMEL antigen or larger fragments thereof.

The second group of peptides, which is preferred, can be presented by MHC molecules (in humans: HLA molecules), they have the potential to induce an immune response, in particular by eliciting a CTL response.

25 In a preferred embodiment, immunogenic peptides which have the ability to elicit a CTL response, are selected from peptides with the sequence set forth in SEQ ID NO: 11, 12, 24, 25 and 26.

To obtain peptides that have the ability to elicit a cellular immune response, the selection of peptide sequences from a given antigen is, in the first place, based on the requirement for such peptide to bind to an MHC molecule present in the repertoire of the patient to be treated. Two classes of MHC molecules are distinguished, class I and class II. Class I molecules consist of a membrane-inserted heavy chain and a non-covalently attached light chain. In their structure, MHC class I molecules resemble a moose's head, the antlers forming a groove which is recognized by the peptide. In humans, HLA-A, B and C are the "classical" MHC class I molecules.

Additional immunogenic peptides may be identified by methods known in the art which rely on the correlation between MHC-binding and CTL induction, e.g. those used by Stauss et al., 1992, who identified candidate T-cell epitopes in human papilloma virus.

Since immunogenic peptides can be predicted based on their "peptide binding motif" synthetic peptides which represent CTL epitopes may be designed and synthesized. Several methods, which are useful in the present invention for designing peptides, have been used to identify CTL epitopes from known protein antigens.

It is well established that every MHC class I allelic product has allele-specific requirements for the peptide ligand that binds to its groove and that it ultimately presents. These requirements were summarized as a motif by Falk et al., 1991. A large number of MHC peptide motifs and MHC ligands have become known to date. A method to search a known protein sequence for epitopes fitting to a given class I molecule, which is based on this knowledge and which may be used in the present invention, was reviewed by Rammensee et al., 1995. It comprises the following steps: first, the protein sequence is screened for stretches fitting to the basic anchor motif (two anchors in most cases), whereby allowance should be made for some variation in peptide lengths as well as in anchor occupancy. If a motif, for example calls for 9mers with Ile or Leu at the end, 10mers with

a fitting C-terminus should be considered as well, and other aliphatic residues at the C-terminus, like Val or Met, should also be considered. In this way, a list of peptide candidates is obtained. These are inspected for having as many non-anchor residues as possible in common with ligand already known, or with the residues listed among the "preferred residues" or "others" on top of each motif (Table, given by Rammensee et al., 1995), for various HLA molecules. Binding assays can be performed at this stage to exclude weak binders which occur frequently among peptides conforming to a basic motif. If a detailed study on peptide binding requirements is available, the candidates can also be screened for non-anchor residues detrimental or optimal for binding (Ruppert et al., 1993). One should keep in mind, however, that pure peptide binding motifs can be misleading in the search for natural ligands, since other constraints, such as enzyme specificity during antigen processing and specificity of transporters or chaperones, are likely to contribute to ligand identity in addition to the MHC binding specificity.

This approach was successfully applied by, inter alia, Kawakami et al., 1995, to identify gp100 epitopes based on known HLA-A2.1 motifs. The validity of the method was confirmed by identifying, in parallel, the epitope regions by using COS cells transfected with cDNA fragments generated by sequential deletion and testing for T-cell reactivity, as described above.

Recently, data bases and prediction algorithms have become available that enable to predict, with great reliability, peptide epitopes that bind to HLA molecules of interest.

Examples for peptide candidates with potential immunogenicity that can be derived from the tumor-associated antigen of the present invention, are the CAMEL-derived peptides with the sequence HLSPDQGRE and LMAQEALAE for HLA-A3 or RMAVPLLR for HLA-A3101. Similarly, other peptides for these or for other alleles can be determined by the method mentioned above.

- The peptide binding can be tested in peptide binding assays. In order to determine the immunogenicity of the selected peptide or peptide equivalent, as defined below, which is the crucial parameter for peptide-based vaccine development and which in most cases strongly correlates with the stability of the peptide-MHC interaction (van der Burg et al., 1996), the methods described by Sette et al., 1994, in combination with quantitative HLA-binding assays, may be used. Alternatively, immunogenicity of the selected peptide may be checked by performing *in vitro* CTL induction by known methods e.g. as described below for *ex vivo* CTL induction.
- 5 Alternatively to peptides derived from the naturally expressed tumor antigens, functional equivalents thereof, i.e. peptides with partially altered sequences or substances mimicking peptides, e.g. "peptidomimetics" or retro-inverso peptides, may be obtained by the following methods:
- 10 To enhance the immunogenicity of the peptides, amino acid substitutions may be introduced at anchor positions to increase peptide MHC class I-binding affinity. The modified peptides are subsequently evaluated for enhanced binding and immunogenicity by screening for recognition by TIL (tumor-infiltrating lymphocytes) and CTL induction as described by Parkhurst et al., 1996, and Bakker et al., 1997.
- 15 Another method useful in the present invention to find more immunogenic peptides by screening peptide libraries with a known CTL was described by Blake et al. 1996; it suggests the use of combinatorial peptide libraries for constructing functional mimics of tumor epitopes recognized by MHC class I-restricted CTLs.
- 20 In principle, the selection of peptides capable of eliciting a cellular immune response is carried out in several steps, as described in WO 97/30721, which disclosure is incorporated herein by reference. In short, the candidates are first tested for their binding ability to an MHC molecule; subsequently good binders are tested for immunogenicity. A general
- 25

strategy for obtaining efficient immunogenic peptides has been described by Schweighoffer, 1997.

The polypeptide of the present invention or immunogenic peptides derived from its sequence, respectively, can be produced recombinantly or by peptide synthesis, as described in WO 96/10413, the disclosure of which is incorporated herein by reference. For recombinant production, a DNA molecule encoding the antigen or the CTL peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, cultivated under conditions suitable for expression, recovered and purified. For chemical synthesis, various conventional techniques may be used, e.g. commercially available automatic synthesizers.

The tumor antigen of the present invention and the immunogenic peptides derived therefrom or the respective peptide equivalents are useful in cancer therapy, e.g. to induce, in the context of the appropriate MHC presenting molecule, an immunological response to tumors which express the corresponding antigen determinants. The induction of CTLs can be accomplished *in vivo* or *ex vivo*.

For *in vivo* induction of CTLs, a pharmaceutical composition comprising the peptide/antigen is administered to an individual suffering from a tumor associated with the respective tumor antigen in an amount sufficient to elicit an effective CTL response to the antigen-bearing tumor. Thus, the present invention provides pharmaceutical compositions for therapeutic treatment which are intended for parenteral, topical, oral or local administration. Preferably, the compositions are for parenteral administration, e.g. for intravenous, subcutaneous, intradermal or intramuscular application. The peptides/antigens are dissolved or suspended in a pharmaceutically acceptable carrier, preferably an aqueous carrier. The composition may contain additional auxiliary substances, e.g. buffering agents, etc. The peptides may be used alone or in combination with adjuvants, e.g. saponins, alum, or, in a particularly preferred embodiment, polycations,

like polyarginine or polylysine. The peptides may also be linked to components assisting CTL priming, e.g. T helper peptides, lipids or liposomes or coadministered with such components or with immunostimulating substances, e.g. cytokines (IL-2, IFN- $\gamma$ ). Methods and compositions for preparing and administering pharmaceutical compositions for therapeutic treatments are described in WO 95/04542 and WO 97/30721 the disclosures of which are herein incorporated by reference.

The immunogenic peptides may also be used to elicit a CTL response *ex vivo*. An *ex vivo* CTL response to a tumor expressing the antigen is induced by incubating a patient's CTL precursor cells together with antigen presenting cells and the immunogenic peptide. The thus activated CTLs are allowed to mature and expand to effector CTLs which are then readministered to the patient. Alternatively, the tumor antigen may be pulsed onto APCs which present MHC class II-reactive peptides (Mayordomo et al., 1995; Zitvogel et al., 1996). A suitable method for loading peptides onto cells, e.g. dendritic cells, is disclosed in WO 97/19169.

The peptides of the invention are preferably applied as a combination of peptides, e.g. different CAMEL-peptides. In an even more preferred embodiment, the peptides of the invention are combined with peptides derived from other tumor antigens, e.g. LAGE-1 and ESO-NY-1. The selection of the peptides is optimized towards covering multiple HLA types in order to be useful for a broad population of patients and/or towards a broad variety of malignancies, which is taken into account by combining peptides from a large variety of tumor antigens. The number of peptides suitable to be combined to yield an efficient therapy may vary within a broad range, e.g. from about 2 to approximately 100.

In a further aspect, the present invention is directed to an isolated DNA molecule with the sequence set forth in SEQ ID NO: 1 encoding CAMEL.

This DNA molecule, which is designated "CAMEL-DNA", contains the ORF-1 of LAGE-1 cDNA which is defined by nucleotides 54 - 336 of the sequence set forth in SEQ ID NO: 3.

5 The CAMEL-DNA of the present invention, or the corresponding RNA, may be used, as an alternative to the use of the protein or the peptide, for cancer immunotherapy. Alternatively to using the natural sequence or fragments thereof, engineered derivatives may be utilized. These include sequences modified to encode (poly)peptides with improved immunogenicity, e.g. taking into account the modifications described above  
10 for the peptides. Another form of modification is the assembly of multiple sequences encoding immunologically relevant peptides in a so-called string-of-beads fashion, as described by Toes et al., 1997. The sequences may also be modified by adding auxiliary coding elements, e.g. targeting functions that ensure more efficient delivery and processing of the  
15 immunogen (e.g. Wu et al., 1995).

The nucleic acid molecules may be delivered either directly or as part of a recombinant virus or bacterium. In principle, any method that is known for gene therapy may be applied for nucleic acid-based cancer immunotherapy, both *in vivo* and *ex vivo*.

20 Examples for *in vivo* delivery are direct injection (injection of "naked" DNA) either intramuscularly or by "gene gun", which has been shown to result in the generation of CTLs against tumor antigens. Examples for recombinant organisms are vaccinia virus, fowlpox virus and adenovirus or *Listeria monocytogenes* (see Coulie, 1997 for a comprehensive review).  
25 Furthermore, synthetic nucleic acid carriers like cationic lipids, microspheres, microbeads, liposomes may be useful for *in vivo* delivery of the sequence encoding respective antigen/peptide. Similarly as for peptides, various auxiliary agents that enhance the immune response may be co-applied, e.g. cytokines, either as proteins or as plasmids encoding  
30 these.

Examples for *ex vivo* delivery are transfection of dendritic cells (Tuting, T., 1997) or other antigen presenting cells which are applied as a cellular cancer vaccine.

5 The present invention is also directed to the use of cells that express the tumor-associated antigen of the invention, either naturally or upon transfection with the respective coding sequence, for the preparation of a tumor vaccine.

In the present invention, it has been shown that CTL clones raised against IL-2 producing melanoma cell line 518/IL-2.14 are reactive against two  
10 alternatively spliced variants of LAGE-1, LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) and NY-ESO-1 (SEQ ID NO: 9). NY-ESO-1 is a recently described tumor antigen, identified by screening a cDNA library of an esophagus carcinoma with autologous patient serum (SEREX-method (Chen et al., 1997)). NY-ESO-1 is expressed in different tumor types but  
15 not in healthy tissues except the testis.

In the present invention, the epitope of specific CTL 1/29 was determined by cDNA expression cloning and a truncated LAGE-1 cDNA clone was found. This truncation led to the identification of the peptide epitope in an  
20 alternative reading frame, since the "normal" translation initiation site of LAGE-1 was absent. However, COS/HLA-A\*0201 cells transfected with full length LAGE-1 or NY-ESO-1 cDNA clones could stimulate the CTL clone to TNF- $\alpha$  production as well. This probably means that two different proteins can be translated from one single mRNA.

NY-ESO-1 also has been described as the target of melanoma-specific  
25 HLA-A\*0201 restricted CTL clones, which recognize an epitope translated in ORF3, located between aa 155 and 167 (Jäger et al., 1998). Therefore, it is very likely that also LAGE-1<sup>S</sup> will be recognized by these clones, but not LAGE-1<sup>L</sup>, since the protein sequence is different at this part of the molecule. The CAMEL-specific CTL clones recognize a peptide in an



alternative reading frame, which is encoded in both LAGE-1 and NY-ESO-1. This means that tumor cells expressing either LAGE-1 or NY-ESO-1 can be recognized by MLMAQEALAFI-specific CTL, which might enlarge the number of tumors that can be treated with immunotherapy based on this peptide.

Brief description of the Figures:

Figure 1: COS-7 transfection experiments with cDNA clone CAMEL and deletion constructs

- a) COS-7 cells were transfected with cDNAs as indicated and tested with CTL 1/29 in a TNF- $\alpha$  release assay.
- b) Deletion constructs of CAMEL cDNA were cotransfected with HLA-A\*0201 cDNA in COS-7, followed by a TNF- $\alpha$  release assay with CTL 1/29. The PCR clones contain the numbers of nucleotides of the CAMEL cDNA as indicated.

Figure 2:

- a) Nucleotide alignment of cDNA clones CAMEL, LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1.
- b) Protein translations of the cDNA clones LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1. The translation of CAMEL is identical to the translation of LAGE-1<sup>S/L</sup> in ORF1. Although ORF3 seems the most putative one, the CTL epitope is encoded in ORF1 (underlined).

Figure 3: Characterisation of peptides recognized by CTL clone 1/29

- a) TNF- $\alpha$  release assay with predicted HLA-A\*0201 binding CAMEL peptides. Peptides as indicated were loaded on BLM, an

HLA-A\*0201<sup>+</sup> melanoma cell line, at a concentration of 10 µg/ml and tested with CTL 1/29 in a TNF-α release assay.

- b) The effects of increasing concentrations of peptides, derived from the major target epitope MLMAQEALAFI on recognition by CTL 1/29.
- 5 Various concentrations of peptides as indicated were loaded on HLA-A\*0201<sup>+</sup> cells and tested in a TNF-α release assay with CTL 1/29.

Figure 4: LAGE-1<sup>SL</sup> and NY-ESO-1 encode the CTL epitope

COS/HLA-A\*0201 cells were transfected with these cDNA clones and reactivity with CTL 1/29 was measured in a TNF-α release assay.

Figure 5: His-tagged CAMEL protein, synthesized in E.coli

Figure 6: Expression of LAGE-1<sup>SL</sup> and NY-ESO-1 in healthy human tissues and melanoma cell lines

- a) Northern Blot analysis of the expression of LAGE-1/NY-ESO-1 in a panel of healthy human tissues as indicated. The Blot was hybridised with <sup>32</sup>P-dCTP-labeled LAGE-1<sup>S</sup> cDNA.
- 15
- b) RT-PCR for LAGE-1 and NY-ESO-1. To discriminate between LAGE-1 and NY-ESO-1 mRNA, the same panel of melanoma cell lines was analysed by RT-PCR with gene-specific primers. Melanoma cell lines as indicated were used as targets in a TNF-α release assay with CTL 1/29.
- 20

Figure 7: Immunohistochemical analysis of CAMEL expression in human tumors

Figure 8: Stabilization of HLA-A2 surface expression by synthetic peptides on T2-cells

## Brief description of the sequences:

- SEQ ID NO: 1: CAMEL (4H8) cDNA sequence and translation
- SEQ ID NO: 2: CAMEL protein sequence
- 5 SEQ ID NO: 3: LAGE-1<sup>S</sup> cDNA sequence and translation
- SEQ ID NO: 4: LAGE-1<sup>S</sup> protein sequence
- SEQ ID NO: 5: LAGE-1<sup>L</sup> cDNA sequence and translation
- SEQ ID NO: 6: LAGE-1<sup>L</sup> protein sequence
- SEQ ID NO: 7: NY-ESO-1 cDNA sequence and translation
- 10 SEQ ID NO: 8: NY-ESO-1 protein sequence
- SEQ ID NO: 9: NY-ESO-1 cDNA and alternative translation
- SEQ ID NO: 10: protein sequence of alternatively translated NY-ESO-1
- SEQ ID NO: 11: peptide sequence of the CAMEL CTL epitope (11-mer)
- SEQ ID NO: 12: peptide sequence of the CAMEL CTL epitope (10-mer)
- 15 SEQ ID NO: 13: oligonucleotide SP6F-pSV
- SEQ ID NO: 14: oligonucleotide R1
- SEQ ID NO: 15: oligonucleotide R2
- SEQ ID NO: 16: oligonucleotide T7R-pSV
- SEQ ID NO: 17: oligonucleotide F3
- 20 SEQ ID NO: 18: oligonucleotide ESO-1B
- SEQ ID NO: 19: oligonucleotide ESO-1A
- SEQ ID NO: 20: oligonucleotide 4H8-A
- SEQ ID NO: 21: oligonucleotide 4H8-C
- SEQ ID NO: 22: oligonucleotide CAMEL-XHO
- 25 SEQ ID NO: 23: oligonucleotide CAMEL-KPN

- SEQ ID NO: 24 peptide CAMEL10
- SEQ ID NO: 25 peptide CAMEL16
- SEQ ID NO: 26 peptide CAMEL17
- SEQ ID NO: 27 tyrosinase peptide
- 5 SEQ ID NO: 28 MAGE-3 peptide

In the Examples, if not stated otherwise, the following materials and methods were used

#### a) Cell cultures

- 10 Melanoma cell lines and COS-7 cells were maintained in DMEM containing 4.5 mM glucose supplemented with 8% FCS, 2 mM L-glutamine, 100 µg/ml of each penicillin and streptomycin. Melanoma cell line 518A2 was established from the dissected metastasis of a male patient in 1985, as described before (Versteeg et al., 1988). An IL-2 producing variant, 518/IL-
- 15 2.14, was obtained by transfection of 518A2 with the IL-2 cDNA (Osanto et al., 1993). Other melanoma cells that were used as targets in TNF-α release assay are FM3.29, FM6, FM28.4 and FM55P (gifts from J. Zeuthen, Denmark), MM127, MM415, MM485 (gifts from N. Hayward, Australia), SK-MEL-23, SK-MEL-29 (obtained from T. Wölfel, Mainz), Mi10221, Mi3046/2,
- 20 NA8, BLM (obtained from M. Visseren, Leiden). EBV-transformed B-LCL and the TNF-α-sensitive WEHI-164 clone 13 (a gift from Dr. P. Coulie, Brussels) were cultured in RPMI-1640, supplemented with L-glutamine and antibiotics as above, and 10% FCS.

- With the IL-2 producing cell line 518/IL-2.14 and autologous peripheral
- 25 blood mononuclear cells (PBMC) a CTL induction was performed, resulting in melanoma-specific HLA-A\*0201 restricted CTL clones (van Elsas et al., 1997). The identification of the epitope of one of these clones, CTL 1/29, is reported here.

b) cDNA expression cloning

A cDNA library of 518/IL-2.14 was constructed in the expression vector pSVsport1 (GIBCO, BRL) using the Superscript Plasmid System (GIBCO, BRL). As to that purpose, poly-A<sup>+</sup> mRNA was isolated using the Fast-Track system (Invitrogen), followed by reverse-transcription with an oligo-dT/NotI primer. Sall adapters were ligated to ds-cDNA and after NotI digestion and size fractionation, cDNA fragments were cloned into the pSVsport1 vector digested with Sall and NotI. After electroporation into ElectroMAX-DH10B (GIBCO, BRL) (following the manufacturers instructions) and selection for ampicillin resistance, 50-100 colonies were pooled for mini DNA isolation (QIAprep 8 plasmid kit, Qiagen). The in this way obtained cDNA pools were transfected in duplicate into COS-7 cells, together with the restriction element HLA-A\*0201 (pBJ1.neo/HLA-A\*0201, (Lin et al., 1990)), using the DEAE-dextran method. Briefly, COS-7 cells were seeded in 96-wells flatbottom plates at  $1.5 \times 10^4$  cells per well in 100  $\mu$ l DMEM, 8% FCS. After 2 hours, medium was replaced by 30  $\mu$ l transfection mixture, containing 100 ng cDNA pool, 100 ng HLA-A\*0201 cDNA, 400 ng/ml DEAE-dextran and 100  $\mu$ M chloroquine in serum free DMEM. Cells were incubated for 4 hours at 37°C and shocked for 2 minutes by the addition of 50  $\mu$ l 10% DMSO in PBS. The shock medium was replaced by 200  $\mu$ l DMEM, 8% FCS, and 48 hours later the cells were used as target cells for CTL in a TNF- $\alpha$  release assay.

c) Deletion constructs

Deletion constructs of cDNA clone 4H8 were obtained by PCR. PCR products were cloned in vector pCR3.uni (TA cloning system, Invitrogen). The constructs pCR-246 and pCR-464 were made with the vector-based forward primer, SP6F-pSV (SEQ ID NO: 13) and the reverse primers in cDNA 4H8, R1 (SEQ ID NO: 14) and R2 (SEQ ID NO: 15) respectively. As a control the complete 679 bp cDNA was cloned by PCR with two primers

on the pSVsport vector, SP6F-pSV (SEQ ID NO: 13) and T7R-pSV (SEQ ID NO: 16), resulting in pCR-679.

d) TNF- $\alpha$  release assay

- CTL reactivity against tumor target cells, transfected COS-7 or peptide loaded cells was measured in a TNF- $\alpha$  release assay. Target cells were seeded in duplicate or triplicate at  $1.5 \times 10^4$  cells per well in a 96-wells flat bottom plate and 1500-2000 CTL were added to each well, in a total volume of 100  $\mu$ l / well (IMDM, supplemented with antibiotics and 5% FCS). After 24 hours of co-culturing of effector and target cells, 50  $\mu$ l out of each well was added to a fresh 96-wells flatbottom plate, containing 50  $\mu$ l ( $4.5 \times 10^4$ ) TNF- $\alpha$ -sensitive WEHI-164 cells per well in IMDM, supplemented with antibiotics, 5% FCS, 2  $\mu$ g/ml Actinomycin D and 40 mM LiCl. A viability staining was performed 24 hours later by the addition of 50  $\mu$ l of 3-(4,5dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) solution (2.5 mg/ml in PBS). After incubation for 2-4 hours at 37°C the OD<sub>550-650</sub> was measured. TNF- $\alpha$  release in pg/ml was calculated from a standard with known TNF- $\alpha$  concentrations.

e) Northern Blot analysis

- To determine expression in healthy tissues a Multiple Tissue Northern Blot was obtained commercially (Clontech). As a probe, LAGE-1 cDNA was used, labeled with  $\gamma$ -<sup>32</sup>P-dCTP by use of the Mega-Prime Labeling kit (Amersham).

f) RT-PCR

- cDNA synthesis was performed using oligo-dT and M-MLV reverse transcriptase (Promega). Primers used for LAGE-1 specific PCR were the F3 (SEQ ID NO: 17) and ESO-1B primer (SEQ ID NO: 18). ESO-1B was also used as a reverse primer in the NY-ESO-1-specific PCR, while ESO-1A (SEQ ID NO: 19) was the forward primer in this reaction (Chen et

al., 1997). Reactions were performed in a Biometra-Uno or -Trio programmed as follows: 5 minutes 95°C, 30 cycles of 1 min. 95°C, 1 min. 58°C, 1 min. 72°C, followed by 10 minutes 72°C.

g) Expression of CAMEL in E. Coli

- 5 A fragment containing the coding sequence of CAMEL was made by PCR with the following primers:

4H8-A: GAAGAACATATGCTGATGGCCCAGGAGGC (SEQ ID NO: 20)

4H8-C: TTAAAGATCTCAGAACCGCCCCTGGTCG (SEQ ID NO: 21)

- This fragment was digested with NdeI and BglII and cloned in the NdeI and BamHI sites of vector pET19b (Novagen, Madison, WI). This vector  
10 contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was transformed into BL21(DE3)pLysS E. coli bacteria (Novagen, Madison, WI). After culturing the bacteria at 30°C until an  $OD_{600} = 0.5$ , IPTG (1 mM) was  
15 added to induce overexpression of the His-tagged CAMEL protein. Samples were taken at 0h and 4h after IPTG induction and lysates of these samples were tested on a Western Blot with the Penta-His Antibody (Qiagen) according to the Western and Colony Blot protocol of the supplier. The His-tagged protein was visualized using the SuperSignal Substrate  
20 system for Western blotting (Pierce, Rockford, US).

h) Preparation of anti-CAMEL antisera

Antibodies against the CAMEL protein were raised by immunizing two rabbits with three synthetic peptides derived from hydrophobic regions of this molecule :

- 25 F4: (K)GAMLA AQERRVPRAAEV(K) (pos. 15-31 of SEQ ID NO: 2)  
A5: (K)GQQGPRGREEAPRGVRM(K) (pos. 36 –52 of SEQ ID NO: 2)  
B5: (K)KRRMEGAPAGPGGR TAA(K) (pos. 58 –73 of SEQ ID NO: 2)

(The lysine residues at both termini enable the peptides to be linked to KLH.)

For rabbit no. 1, 1 mg of each peptide was linked chemically to 2.5 mg of the carrier molecule KLH (Keyhole Limpet Hemocyanin) and after dialyzing, 0.8 mg of this protein in CFA (Complete Freund's Adjuvants) was weekly injected subcutaneously. Another rabbit (no. 2) was injected six times with the three peptides, not linked to KLH, following the scheme of 300 µg s.c. in CFA, 300 µg s.c. in IFA, 4x boost of 150 µg. The reactivity and specificity of the antisera were confirmed in ELISA and Western blot experiments. After four immunizations, antisera of both rabbits were reactive with the recombinant CAMEL protein synthesized in *E.coli*, but differed in their precise epitope: rabbit no. 1 produced antibodies against the CAMEL-B5 peptide, whereas the serum of rabbit no. 2 reacted with peptide F4. The antisera will further be referred to as "antiserum B5" and "antiserum F4".

#### j) Preparation of CAMEL-EGFP fusion proteins

The CAMEL coding sequence was fused to the *Aequorea victoria*-derived Green Fluorescent Protein (GFP). The CAMEL cDNA molecule was cloned into the pEGFP-N1 vector (Clontech), which contains a cDNA encoding the Enhanced, red shifted variant of GFP. In order to clone the cDNA molecule in frame with the EGFP cDNA and unidirectional, two primers were designed. The forward primer designated CAMEL-XHO (TTACTCGAGATGCTGATGGCCCAGG; SEQ ID NO: 22 ) covers the initiation codon ATG and contains an Xho1 site and the reverse primer CAMEL-KPN (AAGGTACCTTGAACCGCCCCTGGTCG; SEQ ID NO: 23) contains a mutation of the stop-codon and a Kpn1 site. The vector carrying the fusion construct was transfected into COS cells by calcium phosphate precipitation, protein lysates of the cells were used for Western blotting using CAMEL antisera against the CAMEL peptides B5 and F4, and anti-EGFP antibodies to detect the fusion protein according to standard protocols.



### Example 1

cDNA clone 4H8 (CAMEL) encodes the target for melanoma-specific CTL1/29

- The antigenic epitope of melanoma-specific CTL 1/29 was identified by the expression of cDNA library 518/IL2.14 and the restriction element HLA-A\*0201 in COS-7 cells, followed by CTL screening in a TNF- $\alpha$  release assay. A positive pool of cDNAs was subcloned and clone 4H8, called CAMEL (SEQ ID NO: 1), was found to stimulate TNF- $\alpha$  release by the CTL to a similar extent as the original 518/IL2.14 cell line (Fig. 1). COS-7 cells or COS-7 cells transfected with HLA-A\*0201 or the 4H8 cDNA only were not recognized. The isolated 4H8 cDNA clone has a 679 bp insert, which shows strong homology with NY-ESO-1 (SEQ ID NO: 7), a tumor antigen originally identified as a target for humoral immune responses by serum screening methods (SEREX) (Chen et al., 1997). Colony hybridization of the cDNA library, using clone 4H8 as a probe resulted in the detection of 2 types of full length clones which were called LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) (Fig. 2a). LAGE-1<sup>L</sup> contains a 229 bp insertion at position 457, which has the consensus sequences for an intron, starting with a 5' GT and ending 3' AG. This indicates alternative splicing of LAGE-1 mRNA. However, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1 cDNA sequence.

### Example 2

The peptide epitope of CTL 1/29 is coded in an alternative reading frame of LAGE-1 or NY-ESO-1

- To identify which peptide was recognized by CTL 1/29, deletion constructs of cDNA 4H8 were transfected in HLA-A\*0201<sup>+</sup> COS-7 cells and tested in a TNF- $\alpha$  release assay. CTL reactivity was measured with all constructs (Fig. 1b), indicating that the epitope was coded within the first 330 bp of

clone 4H8. An HLA-A\*0201 binding motif search was performed on the predicted protein sequence of that region (Drijfhout et al., 1995; D'Amato et al., 1995), presuming that the ATG at position 10 in 4H8 functions as the translation initiation site. Predicted strong binding peptides at regions 1-11, 2-11, 1-9, 10-18, 11-19, 16-25, 17-25, 49-57, 55-63 and 70-78 of the CAMEL protein sequence (Fig. 2b) were added to HLA-A\*0201<sup>+</sup> BLM melanoma cells, and tested for CTL reactivity in a TNF- $\alpha$  release assay (Fig. 3b).

At a peptide concentration of 10  $\mu$ g/ml only the N-terminal 11- and 10-mer peptides (M)LMAQEALAF<sub>L</sub> (SEQ ID NO: 11 and NO: 12) induced preponderant recognition by CTL 1/29 (Fig. 3a), indicating that the epitope recognized by the CTL is located in the first 11 amino acids of the CAMEL-encoded protein. Closer inspection of peptides derived of this N-terminal 11-mer in a peptide concentration dependent TNF- $\alpha$  release assay (Fig. 3b) revealed that the methionine at position 1 as well as the leucine at position 11 are of crucial importance for reconstituting CTL reactivity. Deletion of either of these amino acids leads to an at least 5 decades higher peptide concentration required for comparable TNF- $\alpha$  release. The only other peptide showing weak activity is the 3-11 MAQEALAF<sub>L</sub>. In contrast, the MLMAQEAL<sub>A</sub> has no activity at all (Fig. 3b), suggesting that the C-terminal amino acids FL do significantly contribute to the recognition by the CTL.

### Example 3

#### Comparison of CAMEL, LAGE-1<sup>S/L</sup>, NY-ESO-1

As already mentioned, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1<sup>S</sup> sequence, which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in 4H8 corresponds with the ATG at position 94 of LAGE-1<sup>S</sup>, which is however, not in frame with the first ATG at position 54. Therefore, the protein translated from the 4H8 cDNA clone is different from the putative

LAGE-1 protein, since translation takes place in another reading frame (Fig. 2a and b). 4H8 encodes a protein of 109 amino acids (SEQ ID NO: 2) with a predicted molecular weight of 11.7 kD. The LAGE-1<sup>S</sup> protein translated from the first ATG will be a 180 aa protein of 18.2 kD (SEQ ID NO: 4), while  
 5 the unspliced variant, LAGE-1<sup>L</sup>, encodes a 210 aa protein of 21.1 kD (SEQ ID NO: 6). NY-ESO-1 protein (SEQ ID NO: 8) is probably of the same size as LAGE-1<sup>S</sup>, but differs at 26 amino acids. However, if translation of LAGE-1<sup>S/L</sup> starts at the second ATG, proteins will be translated in another reading frame and are in that case identical to the protein translated from cDNA  
 10 4H8. Alternative translation of NY-ESO-1 (SEQ ID NO: 9 and NO: 10) results in a shorter variant of this protein (58 amino acids), because of an earlier stop codon (Fig. 2b), which differs from the CAMEL protein sequence only in its last 5 amino acids (Fig. 2b).

It was examined whether cells transfected with the complete LAGE-1 (or  
 15 NY-ESO-1) cDNA clones are able to stimulate CTL 1/29. Remarkably, COS/HLA-A\*0201 cells transfected with LAGE-1<sup>S</sup>, the alternatively spliced LAGE-1<sup>L</sup> (as well as with the NY-ESO-1) cDNA are able to stimulate CTL 1/29 (Fig. 4). This indicates that, at least in COS-7 cells, protein translation also starts from the second start codon at nucleotide 94 in  
 20 LAGE-1<sup>S</sup>, notwithstanding the presence of the first ATG at position 54. Also in this case, this results in the "alternative reading frame" peptide, MLMAQEALAF<sub>L</sub>, recognized by CTL 1/29.

#### Example 4

##### Expression of CAMEL in E. Coli

25 To investigate whether CAMEL is indeed translated from the ORF-1 of the CAMEL (4H8) cDNA, the CAMEL cDNA (SEQ ID No: 1) was cloned in a bacterial expression vector (pET19b) (Studier et al., 1990). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was

transformed into E.coli and the bacteria were treated with IPTG to induce expression of the His-tagged CAMEL protein. Extracts were analyzed by Western blotting using the Penta-His antibody. Western blotting of a lysate shows a 15.5 kD protein, only slightly higher than the expected 14.5 kD of the His-tagged CAMEL protein after staining with a anti-His antibody (Fig. 5).

The CAMEL cDNA (SEQ ID No: 1) was cloned in pET19b and expressed in E.Coli. Lanes 1 and 2 represent the samples taken at 0h, lanes 3 and 4 at 4h after induction with IPTG. Because CAMEL might be an unstable protein, induction of protein expression was performed in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of PMSF (a protease inhibitor). At the left the positions of the molecular weight marker proteins are indicated.

#### Example 5

Expression of LAGE-1 and NY-ESO-1 in healthy human tissues and melanoma cell lines

Hybridisation of Multiple Tissue Northern blots containing RNA of healthy human tissues with the LAGE-1<sup>S</sup> cDNA showed high expression in testis and placenta and low, (but clear) expression in heart, skeletal muscle and pancreas (Fig. 6a). The positive signals exist of two bands, probably reflecting LAGE-1<sup>S</sup>/NY-ESO-1 (750 bp) and LAGE-1<sup>L</sup> (1000 bp).

Several melanoma cell lines were tested for expression of LAGE-1 and NY-ESO-1 by(Northern Blot analysis and) RT-PCR (Fig. 6b). Because of the strong homology between both genes, it is not possible to discriminate between LAGE-1 and NY-ESO-1 on Northern Blot. Therefore RT-PCR was performed with specific primers. In most cell lines a correlated expression of LAGE-1 and NY-ESO-1 was found; only cell line FM3.29 had expression of LAGE-1, but was negative for NY-ESO-1. Other cell lines expressed

either both or none of the two genes (Fig. 6b). There was a good correlation between the level of expression and the recognition by CTL 1/29 (Fig. 6b).

#### Example 6

##### 5 Determination of LAGE1/CAMEL expression of in human tumors by RT-PCR

In order to evaluate the percentage of LAGE1/CAMEL positive human tumors, individual tumor tissues from breast or lung cancer patients were subjected to RT-PCR, as described in the Method section f), using the LAGE1-specific primers F3 (SEQ ID NO: 17) and ESO-1B (SEQ ID  
10 NO: 18); reactions were performed in a Perkin Elmer 9600 thermocycler with 35 (instead of 30) cycles.

As shown in Table 1, approximately 50% (3/6) of the tested breast cancer specimens and 80% (10/12) of the lung cancer specimens were shown to be positive for LAGE1/CAMEL mRNA .

Table 1:

<i>Tumor type</i>	<i>#</i>	<i>LAGE1/CAMEL mRNA</i>
breast (ILC)	71526 / 85	-
breast (ILC)	44231 / 95	-
breast (ILC)	73507 / 95	+
breast (IDC)	19837 / 95	++
breast (IDC)	4385 / 95	+
breast (IDC)	48897 / 95	-
lung (AC)	15827 / 97	+
lung (AC)	53934 / 97	++
lung (AC)	67086 / 93	++
lung (AC)	62357 / 96	+
lung (AC)	T63244 / 93	-
lung (AC)	T64360 / 93	+
lung (SCC)	92710 / 96	++
lung (SCC)	53005	++
lung (SCC)	28649 / 97	-
lung (SCC)	16251 / 97	+
lung (SCC)	5063 / 93	++
lung (SCC)	7580 / 97	+
testis (positive control)	(GibcoBRL)	+

### Example 7

#### Immunohistochemical analysis of CAMEL expression in human tumors

As described in Examples 5 and 6, LAGE-1 mRNA was detected in a panel  
5 of tumor cell lines and tumor tissues and in a restricted number of healthy  
tissues by means of RT-PCR and Northern blot experiments. However, it  
remained to be determined whether in cells expressing the LAGE-1 mRNA  
also the alternatively translated CAMEL is produced.

Frozen sections from a panel of different human tumors were analyzed by  
10 immunohistochemistry using a CAMEL-specific rabbit antiserum B5 which  
was affinity-purified against the B5 peptide. (For the preparation of the  
antisera, see h) in the Methods section. The B5 antiserum was used  
because B5 is CAMEL-specific, while the F4 antiserum may also recognize  
an epitope present on a protein expressed from the ORF-1 of NY-ESO-1).  
15 Specificity of the purified serum was demonstrated by peptide ELISA and  
Western blotting against COS cells transfected with a CAMEL-GFP fusion  
protein. Immunohistochemistry was performed using a 3-step avidin-biotin-  
peroxidase staining procedure. The results are summarized in Table 2,  
examples are shown in Figure 7.

Table 2:

Tumor type (n=number of cases)	Percentage of positive tumor cells				
	negative	< 10%	10-40%	41-70%	>70%
Breast AC (n=11)	2	4	1	2	2
Colon AC (n=8)	1	0	1	1	5
Lung AC (n=10)	2	2	2	3	1
Lung SCC (n=9)	4	3	1	1	0
Pancreas AC (n=10)	1	2	0	2	5

AC: adenocarcinoma

SCC: squamous cell carcinoma

5

A total of 48 different specimen was investigated. In the majority of cases (38/48) CAMEL expression was detected. About half of the positive cases showed expression of CAMEL in 40% or more of the tumor cells, in some of the cases close to 100% of the tumor cells showed CAMEL-specific staining (an example is shown Figure 7, Colon AC). In the majority of tumor specimens expression was heterogeneous ranging from less than 10% of positive tumor cells to more than 70% of positive tumor cells (Table 2; examples are shown in Figure 7, arrows indicate positive tumor cell staining).

10

## 15 Example 8

Identification of HLA-A2 binding peptides within the CAMEL ORF

In order to identify further HLA-A2 epitopes besides the CTL-epitope (M)LMAQEALAFI (SEQ ID NO:11 and 12), CAMEL (SEQ ID NO:2) was examined according to the algorithms and motifs published by Parker et al.;



1994, and Rammensee et al., 1995. The result of this analysis indicated that several further peptides within the CAMEL protein have the potential to bind to HLA-A2, and three of these candidates -CAMEL10: FLMAQGAML (SEQ ID NO: 24), CAMEL16: AMLAAQERRV (SEQ ID NO: 25) and  
 5 CAMEL17: MLAAQERRV (SEQ ID NO:26) – were synthesized.

These synthetic peptides were evaluated for their ability to increase surface HLA-A2 expression on the transport defective cell line 174CEM.T2 (Nijman et al., 1993). Briefly,  $5 \times 10^5$  cells/0.2 ml/well were seeded in 96-well V-bottom plates and incubated for 16 hours with increasing amounts  
 10 (0-320 µg/ml) of peptide at 37°C in a humidified atmosphere. HLA-A2 surface expression was measured by FACS analysis (Becton Dickinson) using purified BB7.2 as primary antibody and a goat-anti-mouse IgG RPE conjugate(DAKO) as detection antibody.

As positive controls the known HLA-A2 restricted CTL-epitopes from CAMEL (MLMAQEALAFI, SEQ ID NO:11) or tyrosinase (Wölfel et al., 1994; YMNGTMSQV, SEQ ID NO:27) were applied. Negative controls included an HLA-A1 binding (and therefore irrelevant) peptide from MAGE-3 (Gaugler et al. , 1994; EVDPIGHLY, SEQ ID NO:28) or no peptide at all.

20 The results from these experiments suggest that the nonamer CAMEL10 binds with similar affinity to HLA-A2 as compared to the positive controls used in the assay. The two other peptides (CAMEL16 and CAMEL17) showed only low affinity in this assay. Therefore in particular CAMEL10 represents a potential new HLA-A2 restricted CTL-epitope derived from  
 25 CAMEL protein (FIG. 8).

The testing of the immunogenicity of CAMEL10 and if it represents a naturally processed and presented ligand can be done as described in WO 97/30721 and Schweighoffer, 1997.

## Bibliography

- Bakker, A.B.H., van der Burg, S.H., Huijbens, R.J.F., Drijfhout, J.-W.,  
5 Melief, J.M., Adema, G.J., and Figdor, C.G. (1997). Analogues of CTL epitopes with improved MHC Class-I Binding capacity elicit anti-melanome CTL recognizing the wild-type epitope. *Int. J. Cancer* 70, 302-309.
- Blake, J., Johnston, J.V., Hellström, K.E., Marquardt, H., and Chen, L.  
10 (1996). Use of Combinatorial Peptide Libraries to Construct Functional Mimics of Tumor Epitopes Recognized by MHC Class I-Restricted Cytolytic T Lymphocytes. *J. Exp. Med.* 184, 121-130.
- Chen, Y.T., Scanlan, M.J., Sahin, U., Tureci, O., Gure, A.O., Tsang, S.,  
15 Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L.J. (1997). A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1914-1918.
- 20 Coulie P.G. (1997) Human tumour antigens recognized by T cells: new perspectives for anti cancer vaccines. *Mol. Med. Today* 3, 261-268
- D'Amaro, J., Houbiers, J.G., Drijfhout, J.W., Brandt, R.M., Schipper, R.,  
25 Bavinck, J.N., Melief, C.J., and Kast, W.M. (1995). A computer program for predicting possible cytotoxic T lymphocyte epitopes based on HLA class I peptide-binding motifs. *Hum. Immunol.* 43, 13-18.
- Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K.,  
30 Jackson, V., Hamada, H., Pardoll, D., and Mulligan, R. (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent,

specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* **90**, 3539-3543.

Drijfhout, J.W., Brandt, R.M., D'Amaro, J., Kast, W.M., and Melief, C.J.  
 5 (1995). Detailed motifs for peptide binding to HLA-A\*0201 derived from large random sets of peptides using a cellular binding assay. *Hum. Immunol.* **43**, 1-12.

Falk, K., Rötzschke, O., Stevanović, S., Jung, G., and Rammensee, H-G  
 10 (1991). Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* **351**, 290-296.

Fearon, E.R., Pardoll, D.M., Itaya, T., Golumbek, P., Levitsky, H.I., Simons, J.W., Karasuyama, H., Vogelstein, B., and Frost, P. (1990). Interleukine-2  
 15 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* **60**, 397-403.

Gansbacher, B., Zier, K., Daniels, B., Cronin, K., Bannerji, R., and Gilboa, E. (1990). Interleukin 2 gene transfer into tumor cells abrogates  
 20 tumorigenicity and induces protective immunity. *J. Exp. Med.* **172**, 1217-1224.

Gaugler B, van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethe B, Brasseur F, Boon T (1994) *J. Exp. Med.* **179**:921-30

Jäger, E., Chen, Y.T., Drijfhout, J.W., Karbach, J., Ringhoffer, M., Jäger, D., Arand, M., Wada, H., Noguchi, Y., Stockert, E., Old, L.J., and Knuth, A.  
 25 (1998). Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J. Exp. Med.* **187**,  
 30 265-270.

- Kawakami, Y., Eliyahu, S., Jennings, C., Sakaguchi, K., King, X., Southwood, S., Robbins, P.F., Sette, A., Appella, E., and Rosenberg, S.A. (1995). Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. *J. Immunol.* **154**, 3961-3968.
- 5
- Lethe, B., Lucas, S., Michaux, L., Desmet, C., Godelaine, D., Serrano, A., Deplaen, E. and Boon, T. (1998). LAGE-1, a new gene with tumor specificity. *Int. J. Cancer* **76** (6):903-908.
- 10
- Lin, A.Y., Devaux, B., Green, A., Sagerström, C., Elliott, J.F., and Davis, M.M. (1990). Expression of T Cell Antigen Receptor Heterodimers in a Lipid-Linked Form. *Science* **249**, 677-679.
- 15
- Mayordomo, J.I., Zorina, T., Storkus, W.J., Zitvogel, L., Celluzzi, C., Falo, L.D., Melief, C.J., Ildstad, S.T., Kast, W.M., DeLeo, A.B., and Lotze, M.T. (1995). Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nature Medicine* **1**, 1297-1302.
- 20
- Nijman HW, Houbiers JG, Vierboom MP, van der Burg SH, Drijfhout JW, D'Amato J, Kenemans P, Melief CJ, Kast WM (1993) *Eur. J. Immunol.* **6**: 1215-9
- 25
- Osanto, S., Brouwenstyn, N., Vaessen, N., Figdor, C.G., Melief, C.J., and Schrier, P.I. (1993). Immunization with interleukin-2 transfected melanoma cells. A phase I-II study in patients with metastatic melanoma. *Hum. Gene Ther.* **4**, 323-330.
- 30
- Parker KC, Bednarek MA, Coligan JE (1994), *J. Immunol.* **152**:163

Parkhurst, M.R., Salgaller, M.L., Southwood, S., Robbins, P.F., Sette, A., Rosenberg, S.A., and Kawakami, Y. (1996). Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A\*0201-binding residues. *J. Immunol.* 157, 2539-2548.

5

Rammensee, H.G. and Friede, T. (1995). MHC ligands and peptide motifs: first listing. *Immunogenetics* 41, 179-228.

10 Ruppert, J., Sidney, J., Celis, E., Kubo, R.T., Grey, H.M., and Sette, A. (1993). Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell* 74, 929-937.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning, a Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press).

15

Schweighoffer, T. (1997) Molecular cancer vaccines: tumor therapy using antigen-specific immunizations. *Path. Onc. Res.* 3, 164-176

20 Sette, A., Vitiello, A., Reheman, B., Fowler, P., Nayarsina, R., Kast, W.M., Melief, C.J.M., Oseroff, C., Yuan, L., Ruppert, J., Sidney, J., del Guercio, M.-F., Southwood, S., Kubo, R.T., Chesnut, R.W., Grey, H.M., and Chisari, F.V. (1994). The relationship between class I binding affinity and immunogenicity of potential cytotoxic T Cell epitopes. *J. Immunol.* 153, 5586-5592.

25

Stauss, H.J., Davies, H., Sadovnikova, E., Chain, B., Horowitz., and Sinclair, C. (1992). Induction of cytotoxic T lymphocytes with peptides *in vitro*: Identification of candidate T-cell epitopes in human papilloma virus. *Proc. Natl. Acad. Sci. USA.* 89, 7871-7875.

- Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorf, J.W. (1990).  
Use of T7 RNA polymerase to direct expression of cloned genes. *Methods*  
in *Enzymology* 185, 60-89
- 5   Tepper, R.I., Pattengale, P.K., and Leder, P. (1989). Murine interleukine-4  
displays potent anti-tumour activity *in vivo*. *Cell* 57, 503-512.
- Toes, R.E., Hoeben, R.C., Van der Voort, E., Rensing, M.E., Van-der-Eb,  
A.J. Melief, C.J.M., and Oftringa, R. 1997 Protective anti-tumor immunity  
10   induced by vaccination with recombinant adenoviruses encoding multiple  
tumor-associated cytotoxic T lymphocyte epitopes in a string-of-beads  
fashion. *Proc. Natl. Acad. Sci. U.S.A.* 94 (26):14660-14665
- Tuting, T., DeLeo, A.B., Lotze, M.T., and Storkus, W.J. Genetically modified  
bone marrow-derived dendritic cells expressing tumor-associated viral or  
15   "self" antigens induce antitumor immunity *in vivo*. *Eur. J. Immunol.* 27,  
2702-2707, 1997.
- van der Burg, S.H., et al., (1996), Immunogenicity of peptides bound to  
20   MHC class I molecules depends on the MHC-peptide complex stability.  
*J. Immunol.* 156, 3308-3314
- Van den Eynde, B. and Brichard, V.G. (1995). New tumor antigens  
recognized by T cells. *Current Opinion in Immunology* 7, 674-681.
- 25   van Elsas, A., van der Minne, C.E., Borghi, M., van der Spek, C.W.,  
Braakman, E., Osanto, S., and Schrier, P.I. (1996). CTL Recognition of an  
IL-2 Producing Melanoma Vaccine. In: *Immunology of human melanoma.*  
Tumor-host interaction and immunotherapy, edited by M. Maio, Amsterdam:  
30   IOS, 1996, p. 165-173.

- van Elsas, A., Aarmoudse, C., van der Minne, C.E., van der Spek, C.W., Brouwenstijn, N., Osanto, S., and Schrier, P.I. (1997). Transfection of IL-2 augments CTL response to human melanoma cells *in vitro*: immunological characterization of a melanoma vaccine. *Journal of Immunotherapy* 20, 343-353.
- Versteeg, R., Noordermeer, I.A., Krüse-Wolters, K.M., Ruiter, D.J., and Schrier, P.I. (1988). c-Myc downregulates class I HLA expression in human melanomas. *EMBO J.* 7, 1023-1029.
- Wang, R.F., Johnston, S.L., Zeng, G., Topalian, S.L., Schwartzentruber, D.J., and Rosenberg, S.A. (1998). A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. *J. Immunol.* 161, 3596-3606.
- Wölfel T, van Pel A, Brichard V, Schneider J, Seliger B, Meyer zum Büschenfelde KH, Boon T (1994) *Eur. J. Immunol.* 24:759-64
- Wu, T.C., Guarnieri, F.G., Staveley-O'Carroll, K.F., Viscidi, R.P., Levitsky, H.I., Hedrick, L., Cho, K.R., August, J.T., and Pardoll, D.M. (1995). Engineering an intracellular pathway for major histocompatibility complex class II presentation of antigens. *Proc. Natl. Acad. Sci. U.S.A.* 92(25): 11671-11675.
- Zitvogel, L., Mayordomo, J.I., Tjandrawan, T., DeLeo, A.B., Clarke, M.R., Lotze, M.T., and Storkus, W.J. (1996). Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. *J. Exp. Med.* 183, 87-97.

## Claims

1. Tumor-associated antigen CAMEL which is encoded by the ORF-1 of LAGE-1 cDNA and has the amino acid sequence set forth in SEQ ID NO: 2.
2. The tumor-associated antigen of claim 1 for use in cancer therapy.
3. Immunogenic (poly)peptide derived from the tumor-associated antigen of claim 1.
4. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 11).
5. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 12).
6. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Phe Leu Met Ala Gln Gly Ala Met Leu (SEQ ID NO: 24).
7. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Ala Met Leu Ala Ala Gln Glu Arg Arg Val (SEQ ID NO: 25).
8. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Met Leu Ala Ala Gln Glu Arg Arg Val (SEQ ID NO: 26).
9. An immunogenic peptide of any one of claims 3 to 8 for use in cancer immunotherapy.
10. Pharmaceutical composition containing the tumor-associated antigen CAMEL of claim 1.



11. Pharmaceutical composition containing an immunogenic peptide of any one of claims 3 to 8.
12. Isolated DNA molecule comprising the sequence set forth in SEQ ID NO: 1.
- 5 13. Recombinant DNA molecule comprising the DNA molecule of claim 12.
14. A DNA molecule of claim 12 or 13 for use in cancer immunotherapy.

PCT

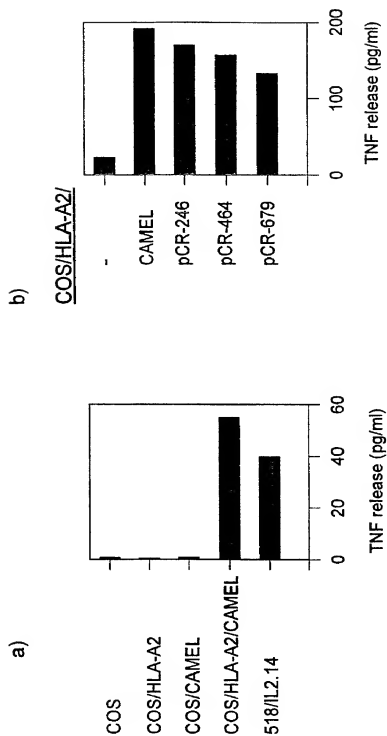
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(74) Agents: LAUDIEN, Dieter; Boehringer Ingelheim GmbH, D-55216 Ingelheim am Rhein (DE) et al.			
(54) Title: CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1			
<p>CAMEL -----CGACGGGCGATGCT 14</p> <p>LAGE-1<sup>3</sup> --ATCCTCGTGGGCCCTGACCTTCTCTGAGAGCCGGGCAGAGGCTCCG 48</p> <p>LAGE-1<sup>4</sup> GCATCCTCGTGGGCCCTGACCTTCTCTGAGAGCCGGGCAGAGGCTCCG 50</p> <p>NY-ESO-1 --ATCCTCGTGGGCCCTGACCTTCTCTGAGAGCCGGGCAGAGGCTCCG 48</p> <p>CAMEL -----CGACGGGCGATGCT 14</p> <p>LAGE-1<sup>3</sup> GAGCCATGCGAGGCCGAGGCCAGGGGCTTCGACGGGCGATGCT 98</p> <p>LAGE-1<sup>4</sup> GAGCCATGCGAGGCCGAGGCCAGGGGCTTCGACGGGCGATGCT 100</p> <p>NY-ESO-1 GAGCCATGCGAGGCCGAGGCCAGGGGCTTCGACGGGCGATGCT 98</p> <p>*****</p> <p>CAMEL GATGGCCAGGAGGCCCTGGCATTCTCTGATGGCCAGGGGGCAATGCTGG 64</p> <p>LAGE-1<sup>3</sup> GATGGCCAGGAGGCCCTGGCATTCTCTGATGGCCAGGGGGCAATGCTGG 148</p> <p>LAGE-1<sup>4</sup> GATGGCCAGGAGGCCCTGGCATTCTCTGATGGCCAGGGGGCAATGCTGG 150</p> <p>NY-ESO-1 GATGGCCAGGAGGCCCTGGCATTCTCTGATGGCCAGGGGGCAATGCTGG 148</p> <p>*****</p> <p>CAMEL CGGCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCGGGGCGCAG 114</p> <p>LAGE-1<sup>3</sup> CGGCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCGGGGCGCAG 198</p> <p>LAGE-1<sup>4</sup> CGGCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCGGGGCGCAG 200</p> <p>LAGE-1<sup>5</sup> CGGCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCGGGGCGCAG 198</p> <p>*****</p>			
(57) Abstract			
<p>The tumor-associated antigen CAMEL and DNA encoding it. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.</p>			

1/15

Fig. 1



2/15  
Fig. 2A

FIGURE 2A)

CAMEL	
LAGE-1 <sup>s</sup>	48
LAGE-1 <sup>L</sup>	50
NY-ESO-1	48
	----
	--ATCCTCGTGGGCCCTGACCTTCTCTGTAGAGCCGGGAGAGGCTCCG
	GCATCCTCGTGGGCCCTGACCTTCTCTGTAGAGCCGGGAGAGGCTCCG
	--ATCCTCGTGGGCCCTGACCTTCTCTGTAGAGCCGGGAGAGGCTCCG
	----
CAMEL	14
LAGE-1 <sup>s</sup>	98
LAGE-1 <sup>L</sup>	100
NY-ESO-1	98
	-----CGACGGGCCG <b>ATG</b> CT
	GAGCC <b>ATG</b> CAGGCCGGAAGGCCAGGCCACAGGGGGTTTCGACGGGCCGATGCT
	GAGCC <b>ATG</b> CAGGCCGGAAGGCCAGGCCACAGGGGGTTTCGACGGGCCGATGCT
	GAGCC <b>ATG</b> CAGGCCGGAAGGCCGCGGGGCACAGGGGGTTTCGACGGGCCGATGCT
	*****
	-----
CAMEL	64
LAGE-1 <sup>s</sup>	148
LAGE-1 <sup>L</sup>	150
NY-ESO-1	148
	GATGGCCCCAGGAGGCCCTGGCATTCTGTATGGCCCCAGGGGGCAATGCTGG
	GATGGCCCCAGGAGGCCCTGGCATTCTGTATGGCCCCAGGGGGCAATGCTGG
	GATGGCCCCAGGAGGCCCTGGCATTCTGTATGGCCCCAGGGGGCAATGCTGG
	GATGGCCCCAGGAGGCCCTGGCATTCTGTATGGCCCCAGGGGGCAATGCTGG
	*****
	-----
CAMEL	114
LAGE-1 <sup>s</sup>	198
LAGE-1 <sup>L</sup>	200
LAGE-1 <sup>L</sup>	198
	CGGCCCCAGGAGAGCGGGGTGCCACGGGGGGCAGAGGTCCTCCCGGGGGCGAG
	CGGCCCCAGGAGAGCGGGGTGCCACGGGGGGCAGAGGTCCTCCCGGGGGCGAG
	CGGCCCCAGGAGAGCGGGGTGCCACGGGGGGCAGAGGTCCTCCCGGGGGCGAG
	CGGCCCCAGGAGAGCGGGGTGCCACGGGGGGCAGAGGTCCTCCCGGGGGCGAG
	*****

Fig. 2A continued

CAMEL	GGGACGAAGGGCCCTCGGGCCCGAGAGGAGGCCCCCGCGGGGTCCGCAT	164
LAGE-1 <sup>s</sup>	GGGACGAAGGGCCCTCGGGCCCGAGAGGAGGCCCCCGCGGGGTCCGCAT	248
LAGE-1 <sup>L</sup>	GGGACGAAGGGCCCTCGGGCCCGAGAGGAGGCCCCCGCGGGGTCCGCAT	250
NY-ESO-1	GGGACGAAGGGCCCTCGGGCCCGAGAGGAGGCCCCCGCGGGGTCCGCAT	248
	*****	
CAMEL	GGCGGTCCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG	214
LAGE-1 <sup>s</sup>	GGCGGTCCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG	298
LAGE-1 <sup>L</sup>	GGCGGTCCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG	300
NY-ESO-1	GGCGGTCCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG	298
	*****	
CAMEL	GCCGACAGCCGCCCTGCTTCAGTTGCAATCACGATGCCTTCTCGTCGC	264
LAGE-1 <sup>s</sup>	GCCGACAGCCGCCCTGCTTCAGTTGCAATCACGATGCCTTCTCGTCGC	348
LAGE-1 <sup>L</sup>	GCCGACAGCCGCCCTGCTTCAGTTGCAATCACGATGCCTTCTCGTCGC	350
NY-ESO-1	GCCGACAGCCGCCCTGCTTCAGTTGCAATCACGATGCCTTCTCGTCGC	348
	*****	
CAMEL	CCATGGAAGCGGAGCTGGTCCGACAGATCCTGTCCCGGGATGCCGCACCT	314
LAGE-1 <sup>s</sup>	CCATGGAAGCGGAGCTGGTCCGACAGATCCTGTCCCGGGATGCCGCACCT	398
LAGE-1 <sup>L</sup>	CCATGGAAGCGGAGCTGGTCCGACAGATCCTGTCCCGGGATGCCGCACCT	400
NY-ESO-1	CCATGGAAGCGGAGCTGGTCCGACAGATCCTGTCCCGGGATGCCGCACCT	398
	*****	

Fig. 2A continued

CAMEL	CTCCCCGACACAGGGGGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT	364
LAGE-1 <sup>s</sup>	CTCCCCGACACAGGGGGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT	448
LAGE-1 <sup>L</sup>	CTCCCCGACACAGGGGGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT	450
NY-ESO-1	CTTCCCGTCCAGGGGTGCTTCTGAAGGACTTCACCTGTGTCGGGCAACAT	448
	** *** ..***** * ***** * ***** * ***** *	
CAMEL	ACTGTTTAT-----	373
LAGE-1 <sup>s</sup>	ACTGTTTAT-----	457
LAGE-1 <sup>L</sup>	ACTGTTTATGTCAGTTCGGGACCAGGACAGGGAAGGCGCTGGCGGATGA	500
NY-ESO-1	ACTGACTAT-----	457
	****. ***	
CAMEL	-----	373
LAGE-1 <sup>s</sup>	-----	457
LAGE-1 <sup>L</sup>	GGGTGGTGGGTGGGGCTGGGATCCGCCTCCCGGAGGGGCAGAAAGCT	550
NY-ESO-1	-----	457
CAMEL	-----	373
LAGE-1 <sup>s</sup>	-----	457
LAGE-1 <sup>L</sup>	AGAGATCTCAGAACACCCAAACACAAAGTCTCAGAACACAGAGACCTGGTAC	600
NY-ESO-1	-----	457

Fig. 2A continued

CAMEL	-----	373
LAGE-1 <sup>s</sup>	-----	457
LAGE-1 <sup>L</sup>	ACCAGGCCCGCCGACCCGAGGGAGCCAGGGAGATGGGTCCAGAGGTG	650
NY-ESO-1	-----	457
CAMEL	-----	385
LAGE-1 <sup>s</sup>	-----CCGACTGACTGC	469
LAGE-1 <sup>L</sup>	-----CCGACTGACTGC	700
NY-ESO-1	TGCGCTTTAATGTGATGTTCTCTGCCCTCACATTTAGCCGACTGACTGC	469
	-----CCGACTGACTGC	
	*****	
CAMEL	TGCAGACCAACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC	435
LAGE-1 <sup>s</sup>	TGCAGACCAACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC	519
LAGE-1 <sup>L</sup>	TGCAGACCAACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC	750
NY-ESO-1	TGCAGACCAACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC	519
	*****	
CAMEL	TTTCCTGTTGATGTGGATCAGCAGTGCTTCTGCCCGTGTTTTGGCT	485
LAGE-1 <sup>s</sup>	TTTCCTGTTGATGTGGATCAGCAGTGCTTCTGCCCGTGTTTTGGCT	569
LAGE-1 <sup>L</sup>	TTTCCTGTTGATGTGGATCAGCAGTGCTTCTGCCCGTGTTTTGGCT	800
NY-ESO-1	TTTCCTGTTGATGTGGATCAGCAGTGCTTCTGCCCGTGTTTTGGCT	569
	*****	

Fig. 2A continued

CAMEL  
LAGE-1<sup>S</sup> CAGGCTCCCTCAGGGCAGAGGGCGCTAAGCCAGACCTGGCGCCCTTCCTA 535  
LAGE-1<sup>L</sup> CAGGCTCCCTCAGGGCAGAGGGCGCTAAGCCAGACCTGGCGCCCTTCCTA 619  
NY-ESO-1 CAGGCTCCCTCAGGGCAGAGGGCGCTAAGCCAGACCTGGCGCCCTTCCTA..850  
\*\*\* CAGCCTCCCTCAGGGCAGAGGGCGCTAAGCCAGACCTGGCGCCCTTCCTA..519  
\*\*\*\*\*

CAMEL  
LAGE-1<sup>S</sup> GGTATGCCTCCTCCCTAGGGAATGGTCCAGCACGAGTGGCCAGTTCA 585  
LAGE-1<sup>L</sup> GGTATGCCTCCTCCCTAGGGAATGGTCCAGCACGAGTGGCCAGTTCA 669  
NY-ESO-1 GGTATGCCTCCTCCCTAGGGAATGGTCCAGCACGAGTGGCCAGTTCA 900  
\*\*\*\*\* GGTATGCCTCCTCCCTAGGGAATGGTCCAGCACGAGTGGCCAGTTCA 669  
\*\*\*\*\*

CAMEL  
LAGE-1<sup>S</sup> TTGTGGGGCCTGATTGTTTGTGCTGGAGGAGGACGGCTTACATGTTG..635  
LAGE-1<sup>L</sup> TTGTGGGGCCTGATTGTTTGTGCTGGAGGAGGACGGCTTACATGTTG..719  
NY-ESO-1 TTGTGGGGCCTGATTGTTTGTGCTGGAGGAGGACGGCTTACATGTTG..950  
\*\*\*\*\* TTGTGGGGCCTGATTGTTTGTGCTGGAGGAGGACGGCTTACATGTTG..719  
\*\*\*\*\*

CAMEL  
LAGE-1<sup>S</sup> TTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA----- 679  
LAGE-1<sup>L</sup> TTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA----- 767  
NY-ESO-1 TTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA----- 993  
\*\*\*\*\* TTCTGTAGAAAATAAAGCTGAGCTACGAAAAA----- 752  
\*\*\*\*\*



Fig. 2B

## Protein Translations

## ORF3

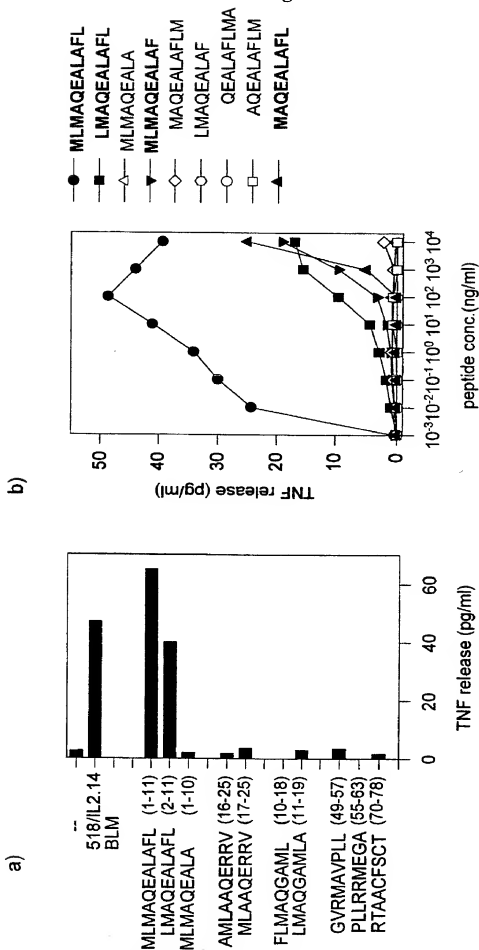
LAGE-1 <sup>S</sup>	MQAEQGQGTGGTGADGPGGGGIPDGGGNAGGPGEAGAT	40
LAGE-1 <sup>L</sup>	MQAEQGQGTGGTGADGPGGGGIPDGGGNAGGPGEAGAT	40
NY-ESO-1	MQAEGRGTGGTGADGPGGGGIPDGGGNAGGPGEAGAT	40
LAGE-1 <sup>S</sup>	GGRGPRGAGAAARASGPRGGAPRGPHGGAASQAQDGRCPCGA	80
LAGE-1 <sup>L</sup>	GGRGPRGAGAAARASGPRGGAPRGPHGGAASQAQDGRCPCGA	80
NY-ESO-1	GGRGPRGAGAAARASGPGGGAPRGPHGGAAS <b>GLNGCC</b> RCGA	80
LAGE-1 <sup>S</sup>	RRPDSRLQLHITMPFFSSPMEAEIVRRILSRDAAPLPRPG	120
LAGE-1 <sup>L</sup>	RRPDSRLQLHITMPFFSSPMEAEIVRRILSRDAAPLPRPG	120
NY-ESO-1	RGPE <b>SRLL</b> <b>EFY</b> LAMP <b>FAT</b> PMFAEL <b>ARRSL</b> AQDA <b>PPL</b> VPVG	120
LAGE-1 <sup>S</sup>	AVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLLM	160
LAGE-1 <sup>L</sup>	AVLKDFTVSGNLLF <b>MSVRDQ</b> REGAG <b>MRVVG</b> LG <b>LSAS</b> P	160
NY-ESO-1	<b>VLLKE</b> FTVSGN <b>IL</b> TIRLTAADHRQLQLSISSCLQQLSLLM	160

Fig. 2B continued

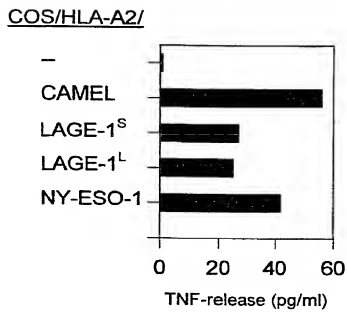
LAGE-1 <sup>S</sup>	WITQCFLPVFLAQAPSGQRR	180
LAGE-1 <sup>L</sup>	<b>EGQKARDLRTPKHKVSEQRPGTGPFPPEGAQGDGCRGVA</b>	200
NY-ESO-1	WITQCFLPVFLAQPPSGQRR	180
LAGE-1 <sup>S</sup>		180 aa, 18.2 kD
LAGE-1 <sup>L</sup>	<b>FNMFSAPHI</b>	210 aa, 21.1 kD
NY-ESO-1		180 aa, 18.2 kD
<b>ORF1</b>		
LAGE-1 <sup>S</sup>	MLMAQEALAFMLAQGAMLAQAQERRVPRAAEVPGAQGGQGP	40
LAGE-1 <sup>L</sup>	MLMAQEALAFMLAQGAMLAQAQERRVPRAAEVPGAQGGQGP	40
NY-ESO-1	<u>MLMAQEALAFMLAQGAMLAQAQERRVPRAAEVPGAQGGQGP</u>	40
LAGE-1 <sup>S</sup>	RGREEAPRGVRMAVPLLRMEGAPAGPGGRTAACFSCTSR	80
LAGE-1 <sup>L</sup>	RGREEAPRGVRMAVPLLRMEGAPAGPGGRTAACFSCTSR	80
NY-ESO-1	RGREEAPRGVRMA <b>ARLQ</b> G	58
LAGE-1 <sup>S</sup>	CLSRPFWKRSWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
LAGE-1 <sup>L</sup>	CLSRPFWKRSWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
NY-ESO-1		58 aa, 6.2 kD

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Fig. 3

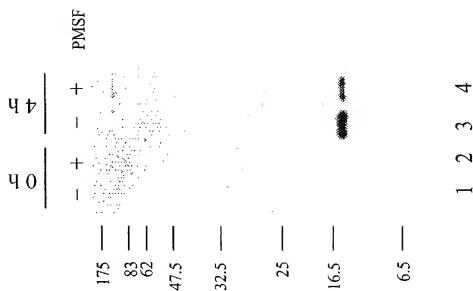


10/15

**Fig. 4**

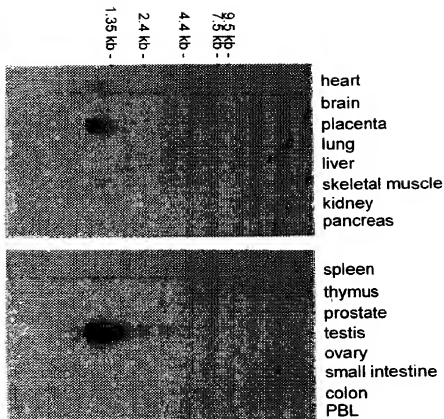
11/15

Fig. 5



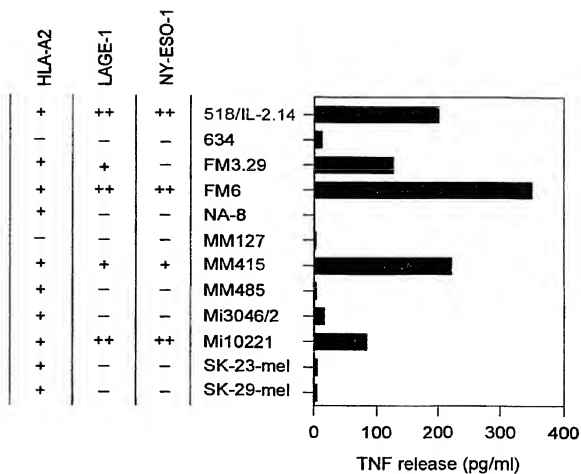
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Fig. 6A



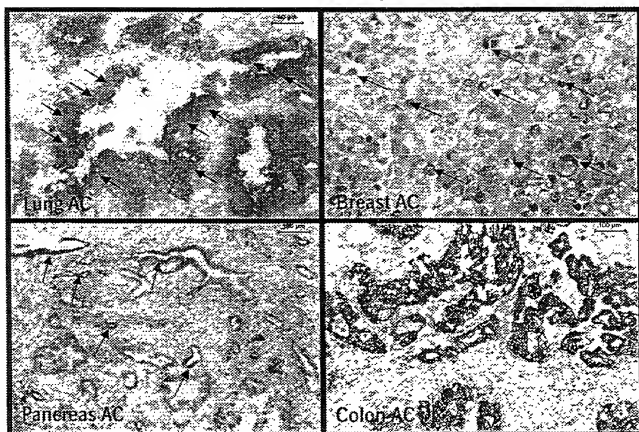
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Fig. 6B



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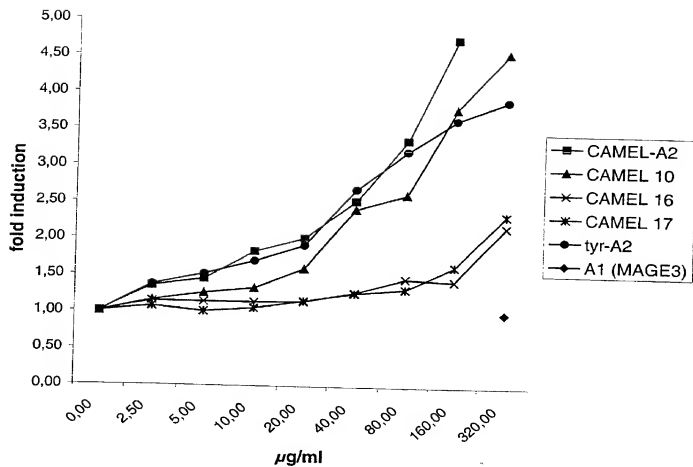
Fig. 7





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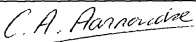
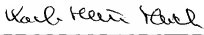
Fig. 8



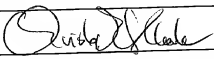


Appl. No. 09/807,512  
Docket No. 0652.2200000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Citizenship	Netherlands		
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Signature of second inventor		22.1.2002	Date
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Citizenship	Netherlands		
Mailing Address	Pr. Alexanderlaan 18, NL-2224 XM Katwijk, Netherlands		
Full name of third inventor	300	<u>Karl-Heinz HEIDER</u>	
Signature of third inventor		26.02.2002	Date
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Appl. No. 09/807,512  
Docket No. 0652.2200000

Full name of fourth inventor	400	Christoph KLADE
Signature of fourth inventor		March 15, 2002 Date
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Mailing Address	Gröhrmühlgasse 1b/17, A-2700 Wr. Neustadt, Austria	

P:\USERS\Schwartz\Cases\0652\2200000\Declaration

## SEQUENCE LISTING

5

## (1) GENERAL INFORMATION:

10

## (i) APPLICANT:

- (A) NAME: Boehringer Ingelheim International GmbH
- (B) STREET: Binger Strasse 173
- (C) CITY: Ingelheim am Rhein
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 55216
- (G) TELEPHONE: 06132/772282
- (H) TELEFAX: 06132/774377

15

20

## (ii) TITLE OF INVENTION: Tumor-associated Antigen

## (iii) NUMBER OF SEQUENCES: 28

25

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

30

35

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

45

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

50

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: homo sapiens
- (F) TISSUE TYPE: Melanoma

55

## (ix) FEATURE:

- (A) NAME/KEY: 3'UTR

(B) LOCATION:340..679

5 (ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..9

10 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION:10..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

15	CGAGCGCGG ATG CTG ATG GCG CAG GAG GCG CTG GCA TTC CTG ATG GCG	48
	Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala	
	1 5 10	
20	CAG GCG GCA ATG CTG GCG GCG CAG GAG AGG CGG GTG CCA CGG GCG GCA	96
	Gln Gly Ala Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala	
	15 20 25	
25	GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG CCT CGG GCG CGA CAG GAG	144
	Glu Val Pro Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu	
	30 35 40 45	
30	GCG CCC CGC GGG GTC CGC ATG GCG GTG CCG CTT CTG CGC AGG ATG GAA	192
	Ala Pro Arg Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu	
	50 55 60	
35	GGT GGC CCT GCG GGG CCA GGA GGC CGG ACA GGC GGC TGC TTC AGT TGC	240
	Gly Ala Pro Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys	
	65 70 75	
40	ACA TCA CGA TGC CTT TCT CGT CGC CCA TGG AAG CGG AGC TGG TCC GCA	288
	Thr Ser Arg Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala	
	80 85 90	
45	GGA TCC TGT CCC GGG ATG CGG CAC CTC TCC CCC GAC CAG GGG CGG TTC	336
	Gly Ser Cys Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe	
	95 100 105	
50	TGA AGGACTTCAC CGTGTCCCGC AACCTACTGT TTATCCGACT GACTGCTGCA	389
	*	
55	110	
	GACGACCGCC AACGCGAGGT CTCACACAGC TCTTGTCCTC ACCAGCTTTC CCGTGTGATG	449
	TGGATCAGCG AGTGCCTTCT GCGCGTGTTT TTGCTCAGG CTCCCTCAGG GCAGAGCGGC	509
	TAGGCGCAGC CTGGCGCGCC TTCTAGGTC ATGCTCTCTC CCTAGGGGA TGGTCCGAC	569
	ACGAGTGGCC AGTTCATGTG GCGCGGCTGA TTGTTGTGTC CTGGAGGAGG AGCGCTTACA	629
	TGTTTGTTC TGTAAGAAAT AAAGCTGAC TACGAAAAA AAAAAAANA	679

## (2) INFORMATION FOR SEQ ID NO: 2:

5

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 109 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

10

- (ii) MOLECULE TYPE: protein

- (xii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
 1                      5                      10                      15  
 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
                     20                      25                      30  
 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
                     35                      40                      45  
 Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu Gly Ala Pro  
                     50                      55                      60  
 Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys Thr Ser Arg  
                     65                      70                      75                      80  
 Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala Gly Ser Cys  
                     85                      90                      95  
 Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe \*  
                     100                      105                      110

35

## (2) INFORMATION FOR SEQ ID NO: 3:

40

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 767 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

45

- (ii) MOLECULE TYPE: cDNA to mRNA

- (iii) HYPOTHETICAL: NO

50

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: homo sapiens  
 (F) TISSUE TYPE: Melanoma

55

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 54..596

## (ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:597..767

## (ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..53

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

5	ATCTCTGCGG GGGCTGAGCT TCTCTCTGAG AGCGGGGAG AGGCTCGGA GGC ATG	56
	Met	
15	1	
	CAG GGC GAA GGC CAG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC	104
	Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
20	5 10 15	
	CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	152
	Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
	20 25 30	
25	CCA GGA GAG GGG GGT GGC ACG GGC GGC AGA GGT CCC CGS GGC CCA GGG	200
	Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
	35 40 45	
30	GCA GCA AGS GGC TGG GGS CGS AGA GGA GGC GGC CGS CGS GGT CGS CAT	248
	Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
35	GGC GGT GGC GCT TCT GGS CAG GAT GGA AGS TGC CCC TGC GGS GGC AGS	296
	Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	
	70 75 80	
40	AGG CGS GAC AGC CGC CTG CTT CAG TTG CAC ATC AGS ATG CCT TTC TCG	344
	Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser	
	85 90 95	
45	TGG CCC ATG GAA GGG GAG CTG CTC GGC AGG ATC CTG TCC CGS GAT GGC	392
	Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	
	100 105 110	
50	GCA CCT CTC CCC CGA CCA GGG GGC GTT CTG AAG GAC TTC ACC GTG TCC	440
	Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	
	115 120 125	
55	GGC AAC CTA CTG TTT ATC CGA CTG ACT GCT CCA GAC CAC CGC CAA CTG	488
	Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu	
	130 135 140 145	
	CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TTG ATG TGG	536
	Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp	
	150 155 160	



	ATC ACG CAG TGC TTT CTG CCC GIG TTT TTG GCT CAG GCT CCC TCA GGG	584
	Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser Gly	
	165 170 175	
5	CAG AGG CCC TAA GGCAGGCTG GGGGGCCTC CTAGGTCATG CCTCTCCCC	636
	Gln Arg Arg *	
	180	
10	TAGGCAATG TOCCAGCAG AGTGGCCAGT TCATGTGGG GGCCTGATG TTGTGCGTG	696
	GAGCAGCAG GCTTACATGT TGTGTCTGT AGAAATATAA GCTGAGCTAC CAAAAAATAA	756
	AAAAAAAAA A	767
15		
	(2) INFORMATION FOR SEQ ID NO: 4:	
20	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 180 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: Linear	
25	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	
30	Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp	
	1 5 10 15	
	Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly	
	20 25 30	
35	Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala	
	35 40 45	
	Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro	
	50 55 60	
40	His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala	
	65 70 75 80	
	Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe	
	85 90 95	
45	Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp	
	100 105 110	
50	Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val	
	115 120 125	
	Ser Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln	
	130 135 140	
55	Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met	
	145 150 155 160	

09/807512

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser  
 165 170 175

5 Gly Gln Arg Arg \*  
 180

10 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 993 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens  
 (F) TISSUE TYPE: Melanoma

(ix) FEATURE:

(A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..55

(ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION:56..688

(ix) FEATURE:

(A) NAME/KEY: 3'UTR  
 (B) LOCATION:689..993

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCATCCCTGGT GGGGCTCGAC CTCCTCTCTG AGAGGCGGGC AGAGGCTGG GAGCC ATG	58
Met	
1	
CAG GGC GAA GGC CAG GGC ACA GGG GGT TGG AGC CGC GAT GCT GAT GGC	106
Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
5 10 15	
CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	154
Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
20 25 30	
CCA GGA GAG GGG GGT GGC AGC GGC GGC AGA GGT CCG CCG GGC CCA GGC	202
Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
35 40 45	
GCA GCA AGG GGC TGG GGG CCG AGA GGA GGC GGC CCG CCG GGT CCG CAT	250

WO 00/23584

7/18

PCT/EP99/07832

	Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
5	GCC GGT GGC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GGC AGG Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	298
	70 75 80	
10	AGG CCG GAC AGC GCG CTG CTT CAG TTG CAC ATC ACG ATG CCT TTC TCG Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser	346
	85 90 95	
15	TGG CCC ATG GAA GCG GAG CTG GTC GCG AGG ATC CTG TCC CGG GAT GGC Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	394
	100 105 110	
20	GCA CCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	442
	115 120 125	
25	GGC AAC CTA CTG TTT ATG TCA GTT CGG GAC CAG GAC AGG GAA GGC GCT Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly Ala	490
	130 135 140 145	
30	GGG CGG ATG AGG GTG GTG GGT TGG GCG CTG GGA TCC GGC TCC CCG GAG Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro Glu	538*
	150 155 160	
35	GGG CAG AAA GCT AGA GAT CTC AGA ACA CCC AAA CAC AAG GTC TCA GAA Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser Glu	586
	165 170 175	
40	CAG AGA CCT GGT ACA CCA GGC CCG CCG CCA CCC GAG GGA GGC CAG GGA Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Pro Glu Gly Ala Gln Gly	634
	180 185 190	
45	GAT GGG TGC AGA GGT GTC GGC TTT AAT GTG ATG TTC TCT GGC CCT CAC Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro His	682
	195 200 205	
50	ATT TAG CCGACTGACT GCTGCGAGAC ACGGCGACT CGAGCTCTCC ATCAGCTCCT Ile *	738
	210	
55	GCTCCAGCA GCTTTCCCG TTGATGIGGA TCAGCAGTG CTTCTCGCC GIGTTTGGG CTCAGGCTCC CTCAGGCGAG AGGCGCTAAG CCCAGGCTCG CGGCGCTTCC TAGGTCATGC CTCTCCCTT AGGGATGGT CCCAGCAAGA GTGGCCAGTT CATGTGCGGG GCGTGAATGT	798
		858
		918
	TTGTGCTGG AGGAGGAGCG CTATCATGTT TGTTCCTGTA GAAATATAG CTAGCTCAAG	978
	AAAAAAAAAAAA	993

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 210 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

10 Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp  
 1 5 10 15  
 Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
 20 25 30  
 15 Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
 35 40 45  
 Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
 50 55 60  
 20 His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
 65 70 75 80  
 25 Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
 85 90 95  
 Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
 100 105 110  
 30 Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
 115 120 125  
 Ser Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly  
 130 135 140  
 35 Ala Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro  
 145 150 155 160  
 40 Glu Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser  
 165 170 175  
 Glu Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Glu Gly Ala Gln  
 180 185 190  
 45 Gly Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro  
 195 200 205  
 50 His Ile \*  
 210

55

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 752 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens

15 (ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..53

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION:54..596

20 (ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 (B) LOCATION:597..752

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

30 ATCTCTGTGG GGGCTGACT TCTCTCTGAG AGCGCGCGAG AGGCTCCGGA GCC ATG 56  
 Met  
 1

35 CAG GCC GAA GGC CGG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC 104  
 Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly  
 5 10 15

CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC 152  
 Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly  
 20 25 30

CCA GGA GAG CGG GGT GGC ACG GGC GGC ACA GGT CCG CGG GGC CCA GGG 200  
 Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly  
 35 40 45

GCA GCA AGG GGC TGG GGG CCG GGA GGA GGC GGC CCG CGG GGT CCG CAT 248  
 Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro His  
 50 55 60 65

GGC GGC GGC GCT TCA GGG CTG AAT GGA TGC TGC AGA TGC GGG GGC AGG 296  
 Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala Arg  
 70 75 80

GGG CCG GAG AGC CGC CTG CTT GAG TTC TAC CTC GCC AAG OCT TTC GCG 344  
 Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe Ala  
 85 90 95

ACA CCC ATG GAA GCA GAG CTG GGC CGC AGG AGC CTG GGC CAG GAT GGC 392

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	Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp Ala	
	100 105 110	
5	CCA CCG CTT CCC GTG CCA GCG GTG CTT CTG AAG GAG TTC ACT GTG TCC Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val Ser	440
	115 120 125	
10	GGC AAC ATA CTG ACT ATC CGA CTG ACT GCT GCA GAC CAC CCG CAA CTG Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu	488
	130 135 140 145	
15	CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TIG ATG TGG Gln Leu Ser Ile Ser Ser Cys Leu Gln Leu Ser Leu Leu Met Trp	536
	150 155 160	
	ATC AGC CAG TGC TTT CTG CCC GTG TTT TIG GCT CAG CCT CCC TCA GGG Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser Gly	584
	165 170 175	
20	CAG AGG CCG TAA GCGGAGGCTG GCGGCGGCTC CTAGGTCATG CCTCTCTCCC Gln Arg Arg *	636
	180	
25	TAGGGAATGG TCCGAGAGG AGTGGCGAGT TCATTGTCGG GCGGTGATG TTTTGCGTG GAGGAGGAG GCTTACATGT TTGTTCTGT AGAAATATA ACTGAGCTAC GAAAA	696
		752

30

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 180 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

	Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp	
	1 5 10 15	
45	Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly	
	20 25 30	
	Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala	
	35 40 45	
50	Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro	
	50 55 60	
55	His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Arg Cys Gly Ala	
	65 70 75 80	
	Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe	
	85 90 95	

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11/18

	Ala Thr Pro Met	Glu Ala Glu Leu Ala Arg Arg Ser	Leu Ala Gln Asp	
	100	105	110	
5	Ala Pro Pro Leu Pro Val	Pro Gly Val Leu Leu Lys	Glu Phe Thr Val	
	115	120	125	
	Ser Gly Asn Ile Leu Thr	Ile Arg Leu Thr Ala Ala Asp His	Arg Gln	
10	130	135	140	
	Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met			
	145	150	155	160
15	Trp Ile Thr Gln Cys Phe Leu Pro Val	Phe Leu Ala Gln Pro Pro Ser		
	165	170	175	
	Gly Gln Arg Arg *			
	180			

20

(2) INFORMATION FOR SEQ ID NO: 9:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 752 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: homo sapiens

40 (ix) FEATURE:  
(A) NAME/KEY: 5'UTR  
(B) LOCATION:1..93

45 (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 94..270

(ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 (B) LOCATION: 271..752

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

55 ATCTCTGTGG GGCCTGACCT TCTCTCTGAG AGCGGGGAG AGGCTGGGA GGCATGACGG 60  
CGCAGGGCG GGGCAGAGG GGTGTGACGG GCG ATG CTG ATG GCG CAG GAG GCG 114  
Met Leu Met Ala Gln Glu Ala

	1	5	
5	CTG GCA TTC CTG ATG GGC CAG GGG GCA ATG CTG GGG GGC CAG GAG AGG Leu Ala Phe Leu Met Ala Gln Gly Ala Met Leu Ala Ala Gln Glu Arg	10 15 20	162
10	CGG GTG CCA CGG GGG GCA GAG GTC CCC GGG GGG CAG GGG CAG CAA GGG Arg Val Pro Arg Ala Ala Glu Val Pro Gly Ala Gln Gly Gln Gln Gly	25 30 35	210
15	CGT CGG GGC CGG GAG GAG GGG CCC GGC GGG GTC GGC ATG GGG GGG CGG Pro Arg Gly Arg Glu Glu Ala Pro Arg Gly Val Arg Met Ala Ala Arg	40 45 50 55	258
20	CIT CAG GGC TGA ATGGATGCTG CAGATGGGG GGCAGGGGGC CGGAGAGGGC Leu Gln Gly *		310
25	CCGCTTGAG TTCTACTGTG CCATGCTTTT CGGACACCC ATGGAAGCAG AGCTGGGGGG CAGGAGGCTG GGCAGGATG CCCCACGCTT TCCCGTGCCA GGGTGCTTC TGAAGAGATT CAGTGTGTC GGCACATAC TGACTATCGG ACTGACTGCT GCGACACCC GGCACATGCA		370 430 490
30	GCTCTCATC AGCTCTGTC TCCAGGAGCT TTCCCTGTG ATGTGATCA CGCATGCTT TCTGGGGTG TTTTGTGCTC AGGCTGCTC AGGGCAGAG GGCATAGGCC AGGCTGGGGC CCCTCCAGG GTCATGCTTC CTCCCTAGG GATGTGTCC AGCAGAGTG GGCAGTTCAT TGTGGGGGC TGATGTGTG TGGCTGGAGG AGGAGGGCTT ACATGTTTGT TTCTGTAGAA AATAAACTG AGCTAGGAA AA		550 610 670 730 752

## (2) INFORMATION FOR SEQ ID NO: 10:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 58 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
 1 5 10 15  
 5 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
 20 25 30  
 10 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
 35 40 45  
 Gly Val Arg Met Ala Ala Arg Leu Gln Gly \*  
 50 55  
 15

2) INFORMATION FOR SEQ ID NO: 11:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 11 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 25 (ii) MOLECULE TYPE: protein  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:  
 30 Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10....

35 2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 amino acids  
 (B) TYPE: amino acid  
 40 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:  
 45 Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10.

50

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:  
 55 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

09/807512

(ii) MOLECULE TYPE: synthetic DNA

5 GGTCACACTA TAGAGGTAC G 21

10 (2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: synthetic DNA  
20 TGATGTGCAA CTGAGGAGG.....20

25 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: synthetic DNA  
35 GCACGCGG ATCACATCA A 21

40 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: synthetic DNA  
50 CGACTACIA TAGGGAGCA G 21

## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: synthetic DNA

GCACATCAGC ATGCCTTCTC CGTGG

25

15

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 25 (ii) MOLECULE TYPE: synthetic DNA

CACACAAGC TTGGCTAGC GCCTCGGC TG.....32

30

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 40 (ii) MOLECULE TYPE: synthetic DNA

CACACAGAT CCATGGATGC TGCAGATGC.....30

45

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 55 (ii) MOLECULE TYPE: synthetic DNA

GAAGACATA TGCTGATGC CCAGGAGC

29

## (2) INFORMATION FOR SEQ ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: synthetic DNA

TTAAAGATCT CAGACCGCC CTTGTCG

28

## (2) INFORMATION FOR SEQ ID NO: 22:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: synthetic DNA

ttactogaga tgctgatgc ccagg.....25

## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: synthetic DNA

aaggtaacctt gaacggcccc tggctg .....26

## 2) INFORMATION FOR SEQ ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Phe Leu Met Ala Gln Gly Ala Met Leu  
1 5 9

5

2) INFORMATION FOR SEQ ID NO: 25:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Ala Met Leu Ala Ala Gln Glu Arg Arg Val  
1 5 10

25

2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Leu Ala Ala Gln Glu Arg Arg Val  
1 5 9

40

2) INFORMATION FOR SEQ ID NO: 27:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Tyr Tyr Met Asn Gly Thr Met Ser Gln Val  
1 5 10

55

## 2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Glu Val Asp Pro Ile Gly His Leu Tyr  
1 5 9

15

-1-

## SEQUENCE LISTING

<110> Schrier, Peter I.  
Aarnoudse, Corlien  
Heider, Karl-Heinz  
Klade, Christoph

<120> Camel, An Alternative Translation Product of the Tumor  
Antigen-Lage 1

<130> 0652.2200000

<140> 09/807,512

<141> 2001-04-16

<150> PCT/EP99/07832

<151> 1999-10-15

<150> EP 98119583.7

<151> 1998-10-16

<160> 28

<170> PatentIn Ver. 2.1

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<212> DNA

<213> Homo sapiens

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<221> 3'UTR

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<221> 5'UTR

<222> (1)..(9)

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<221> CDS

<222> (10)..(339)

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Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala	
1 5 10	

CAG GGG GCA ATG CTG GCG GCC CAG GAG AGG CGG GTG CCA CGG GCG GCA	96
Gln Gly Ala Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala	
15 20 25	

GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG CCT CGG GGC CGA GAG GAG	144
Glu Val Pro Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu	
30 35 40 45	

GCG CCC CGC GGG GTC CGC ATG GCG GTG CCG CTT CTG CGC AGG ATG GAA	192
Ala Pro Arg Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu	

-2-

50

55

60

GGT GCC CCT GCG GGG CCA GGA GGC CGG ACA GCC GCC TGC TTC AGT TGC 240  
 Gly Ala Pro Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys  
                     65                    70                    75

ACA TCA CGA TGC CTT TCT CGT CGC CCA TGG AAG CGG AGC TGG TCC GCA 288  
 Thr Ser Arg Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala  
                     80                    85                    90

GGA TCC TGT CCC GGG ATG CCG CAC CTC TCC CCC GAC CAG GGG CGG TTC 336  
 Gly Ser Cys Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe  
                     95                    100                    105

TGA AGGACTTCAC CGTGCCGGC AACCTACTGT TTATCCGACT GACTGCTGCA 389

GACCACCGCC AACGTCAGCT CTCCTATCAGC TCCTGTCTCC AGCAGCTTTC CCTGTGTATG 449

TGGATCACGC AGTGCTTTCT GCCCGTGTTT TTGGCTCAGG CTCCTCAGG GCAGAGGCGC 509

TAAGCCCAGC CTGGCGCCCC TTCCTAGGTC ATGCCTCCTC CCCTAGGGAA TGTGTTCCAGC 569

ACGAGTGGCC AGTTTCATTGT GGGGGCCTGA TTGTTGTGTC CTGGAGGAGG ACGGCTTACA 629

TGTTTGTTC TGTAGAAAAT AAAGCTGAGC TACGAAAAA AAAAAAAAAA 679

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Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
   1                    5                    10                    15

Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
                     20                    25                    30

Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
                     35                    40                    45

Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu Gly Ala Pro  
                     50                    55                    60

Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys Thr Ser Arg  
                     65                    70                    75                    80

Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala Gly Ser Cys  
                     85                    90                    95

Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe  
                     100                    105

<210> 3



-3-

<211> 767  
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Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
5 10 15	
CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	152
Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
20 25 30	
CCA GGA GAG GCG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGG	200
Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
35 40 45	
GCA GCA AGG GCC TCG GGG CCG AGA GGA GGC GCC CCG CGG GGT CCG CAT	248
Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
50 55 60 65	
GGC GGT GCC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GCC AGG	296
Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	
70 75 80	
AGG CCG GAC AGC CGC CTG CTT CAG TTG CAC ATC ACG ATG CTT TTC TCG	344
Arg Pro Asp Ser Arg Leu Leu Gln His Ile Thr Met Pro Phe Ser	
85 90 95	
TCG CCC ATG GAA GCG GAG CTG GTC CGC AGG ATC CTG TCC CGG GAT GCC	392
Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	
100 105 110	
GCA CCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC	440
Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	
115 120 125	
GGC AAC CTA CTG TTT ATC CGA CTG ACT GCT GCA GAC CAC CGC CAA CTG	488
Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu	
130 135 140 145	
CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TTG ATG TGG	536
Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp	
150 155 160	

-4-

ATC ACG CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG GCT CCC TCA GGG 584  
 Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser Gly  
 165 170 175

CAG AGG CGC TAA GCCCAGCCTG GCGCCCTTC CTAGGTCATG CCTCCTCCCC 636  
 Gln Arg Arg  
 180

TAGGGAATGG TCCCAGCACG AGTGGCCAGT TCATTGTGGG GGCCTGATTG TTTGTGCGTG 696

GAGGAGGACG GCTTACATGT TTGTTTCTGT AGAAAATAAA GCTGAGCTAC GAAAAAATAA 756

AAAAAAAAA A 767

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 1 5 10 15

Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
 20 25 30

Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
 35 40 45

Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
 50 55 60

His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
 65 70 75 80

Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
 85 90 95

Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
 100 105 110

Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
 115 120 125

Ser Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
 130 135 140

Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
 145 150 155 160

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser  
 165 170 175

Gly Gln Arg Arg  
 180

-5-

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Met  
1

CAG GCC GAA GGC CAG GGC ACA GGG GGT TCG ACG GGC GAT GCT GAT GGC 106  
Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly  
5 10 15

CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC 154  
Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly  
20 25 30

CCA GGA GAG GCG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGG 202  
Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly  
35 40 45

GCA GCA AGG GCC TCG GGG CCG AGA GGA GGC GCC CCG CGG GGT CCG CAT 250  
Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His  
50 55 60 65

GGC GGT GCC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GCC AGG 298  
Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg  
70 75 80

AGG CCG GAC AGC CGC CTG CTT CAG TTG CAC ATC ACG ATG CCT TTC TCG 346  
Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser  
85 90 95

TCG CCC ATG GAA GCG GAG CTG GTC CGC AGG ATC CTG TCC CGG GAT GCC 394  
Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala  
100 105 110

GCA CCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC 442  
Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser  
115 120 125

GGC AAC CTA CTG TTT ATG TCA GTT CGG GAC CAG GAC AGG GAA GGC GCT 490  
Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly Ala  
130 135 140 145

GGG CGG ATG AGG GTG GTG GGT TGG GGG CTG GGA TCC GCC TCC CCG GAG 538  
Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro Glu  
150 155 160

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GGG CAG AAA GCT AGA GAT CTC AGA ACA CCC AAA CAC AAG GTC TCA GAA 586  
 Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser Glu  
 165 170 175

CAG AGA CCT GGT ACA CCA GGC CCG CCG CCA CCC GAG GGA GCC CAG GGA 634  
 Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Glu Gly Ala Gln Gly  
 180 185 190

GAT GGG TGC AGA GGT GTC GCC TTT AAT GTG ATG TTC TCT GCC CCT CAC 682  
 Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Ser His  
 195 200 205

ATT TAG CCGACTGACT GCTGCAGACC ACCGCCAACT GCAGCTCTCC ATCAGCTCCT 738  
 Ile  
 210

GTCTCCAGCA GCTTTCCTTG TTGATGTGGA TCACGCAGTG CTTTCTGCCC TGTTTITTTGG 798

CTCAGGCTCC CTCAGGCGAG AGGCGCTAAG CCCAGCCTGG CGCCCTTCC TAGGTCATGC 858

CTCTCCCTCC AGGGAATGGT CCCAGCACGA GTGGCCAGTT CATTGTGGGG GCCTGATTGT 918

TTGTCGCTGG AGGAGGACGG CTTACATGTT TGTTTCTGTA GAAAATAAAG CTGAGCTACG 978

AAAAAAAAAAAA 993

<210> 6  
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 <212> PRT  
 <213> Homo sapiens

<400> 6

Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp  
 1 5 10 15

Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
 20 25 30

Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
 35 40 45

Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
 50 55 60

His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
 65 70 75 80

Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
 85 90 95

Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
 100 105 110

Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
 115 120 125

Ser Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly  
 130 135 140

Ala Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro  
 145 150 155 160

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Glu Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser  
 165 170 175

Glu Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Pro Glu Gly Ala Gln  
 180 185 190

Gly Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro  
 195 200 205

His Ile  
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<210> 7  
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<220>  
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 <222> (597)..(752)

<400> 7

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 Met  
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CAG GCC GAA GGC CGG GGC ACA GGG GGT TCG ACG GGC GAT GCT GAT GGC 104  
 Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly  
 5 10 15

CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC 152  
 Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly  
 20 25 30

CCA GGA GAG CGG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGC 200  
 Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly  
 35 40 45

GCA GCA AGG GCC TCG GGG CCG GGA GGA GGC GCC CCG CGG GGT CCG CAT 248  
 Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro His  
 50 55 60 65

GGC GGC GCG GCT TCA GGG CTG AAT GGA TGC TGC AGA TGC GGG GCC AGG 296  
 Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Arg Cys Gly Ala Arg  
 70 75 80

GGG CCG GAG AGC CGC CTG CTT GAG TTC TAC CTC GCC ATG CCT TTC GCG 344  
 Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe Ala

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85	90	95	
ACA CCC ATG GAA GCA GAG CTG GCC CGC AGG AGC CTG GCC CAG GAT GCC			392
Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp Ala			
100	105	110	
CCA CCG CTT CCC GTG CCA GGG GTG CTT CTG AAG GAG TTC ACT GTG TCC			440
Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val Ser			
115	120	125	
GGC AAC ATA CTG ACT ATC CGA CTG ACT GCT GCA GAC CAC CGC CAA CTG			488
Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu			
130	135	140	145
CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TTG ATG TGG			536
Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp			
150	155	160	
ATC ACG CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG CCT CCC TCA GGG			584
Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser Gly			
165	170	175	
CAG AGG CGC TAA GCCCAGCCTG GCGCCCTTC CTAGGTCATG CCTCCTCCCC			636
Gln Arg Arg			
180			
TAGGGAATGG TCCCAGCACG AGTGGCCAGT TCATTGTGGG GGCTGATTG TTGTGCGTG			696
GAGGAGGACG GCTTACATGT TTGTTTCTGT AGAAAATAAA ACTGAGCTAC GAAAAA			752
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<212> PRT			
<213> Homo sapiens			
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Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp			
1	5	10	15
Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly			
20	25	30	
Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala			
35	40	45	
Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro			
50	55	60	
His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala			
65	70	75	80
Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe			
85	90	95	
Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp			
100	105	110	
Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val			
115	120	125	

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Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
130 135 140

Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
145 150 155 160

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser  
165 170 175

Gly Gln Arg Arg  
180

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<210> 9
<211> 752
<212> DNA
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<220>  
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<222> (1)..(93)

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CCGAAGGCCG GGGCACAGGG GGTTCGACGG GCG ATG CTG ATG GCC CAG GAG GCC 114  
Met Leu Met Ala Gln Glu Ala  
1 5

CTG GCA TTC CTG ATG GCC CAG GGG GCA ATG CTG GCG GCC CAG GAG AGG 162  
Leu Ala Phe Leu Met Ala Gln Gly Ala Met Leu Ala Ala Gln Glu Arg  
10 15 20

CGG GTG CCA CGG GCG GCA GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG 210  
Arg Val Pro Arg Ala Ala Glu Val Pro Gly Ala Gln Gly Gln Gln Gly  
25 30 35

CCT CGG GGC CGG GAG GAG GCG CCC CGC GGG GTC CGC ATG GCG GCG CGG 258  
Pro Arg Gly Arg Glu Glu Ala Pro Arg Gly Val Arg Met Ala Ala Arg  
40 45 50 55

CTT CAG GGC TGA ATGGATGCTG CAGATGCGGG GCCAGGGGGC CGGAGAGCCG 310  
Leu Gln Gly

CCTGCTTGAG TTCTACCTCG CCATGCCTTT CGCGACACCC ATGGAAGCAG AGCTGGCCCG 370

CAGGAGCCTG GCCCAGGATG CCCCACCGCT TCCCGTGCCA GGGGTGCTTC TGAAGGAGTT 430

CACTGTGTCC GGCAACATAC TGACTATCCG ACTGACTGCT GCAGACCACC GCCAACTGCA 490

GCTCTCCATC AGCTCCTGTC TCCAGCAGCT TTCCCTGTTG ATGTGGATCA CGCAGTGCTT 550

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TCTGCCCGTG TTTTGGCTC AGCCTCCCTC AGGGCAGAGG CGCTAAGCCC AGCCTGGCGC 610  
 CCCTTCCTAG GTCATGCCTC CTCCTTAGG GAATGGTCCC AGCAGAGTG GCCAGTTCAT 670  
 TGTGGGGGCC TGATTGTTG TCGCTGGAGG AGGACGGCTT ACATGTTTGT TTCTGTAGAA 730  
 AATAAACTG AGCTACGAAA AA 752

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 <212> PRT  
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<400> 10

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
 1 5 10 15  
 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
 20 25 30  
 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
 35 40 45  
 Gly Val Arg Met Ala Ala Arg Leu Gln Gly  
 50 55

<210> 11  
 <211> 11  
 <212> PRT  
 <213> Homo sapiens

<400> 11

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10

<210> 12  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<400> 12

Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10

<210> 13  
 <211> 21  
 <212> DNA  
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<220>  
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<400> 13  
GGTGACACTA TAGAAGGTAC G 21

<210> 14  
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TGATGTGCAA CTGAAGCAGG 20

<210> 15  
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GCACTGCGTG ATCCACATCA A 21

<210> 16  
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<400> 16  
CGACTCACTA TAGGGAGAGA G 21

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<400> 17  
GCACATCAG ATGCCTTTCT CGTCG 25

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<210> 18  
<211> 32  
<212> DNA  
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<400> 18

CACACAAAGC TTGGCTTAGC GCCTCTGCCC TG 32

<210> 19  
<211> 30  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: Primer

<400> 19

CACACAGGAT CCATGGATGC TGCAGATGCG 30

<210> 20  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer

<400> 20

GAAGAACATA TGCTGATGGC CCAGGAGGC 29

<210> 21  
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<223> Description of Artificial Sequence: Primer

<400> 21

TTAAAGATCT CAGAACCGCC CCTGGTCG 28

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<220>  
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25

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<400> 23

AAGGTACCTT GAACCGCCCC TGGTCG

26

<210> 24  
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<212> PRT  
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<400> 24

Phe Leu Met Ala Gln Gly Ala Met Leu  
1 5

<210> 25  
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<212> PRT  
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<400> 25

Ala Met Leu Ala Ala Gln Glu Arg Arg Val  
1 5 10

<210> 26  
<211> 9  
<212> PRT  
<213> Homo sapiens

<400> 26

Met Leu Ala Ala Gln Glu Arg Arg Val  
1 5

<210> 27  
<211> 10  
<212> PRT  
<213> Homo sapiens

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&lt;400&gt; 27

Tyr	Tyr	Met	Asn	Gly	Thr	Met	Ser	Gln	Val
1				5					10

&lt;210&gt; 28

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 28

Glu	Val	Asp	Pro	Ile	Gly	His	Leu	Tyr
1				5				